TO STUDY THE SEROPREVALENCE OF HEPATITIS E VIRUS INFECTION IN ASYMPTOMATIC PREGNANT MOTHERS ATTENDING ROUTINE ANTENATAL CHECK UP IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY

In partial fulfilments of the regulation

for the award of the degree

M.D.(MICROBIOLOGY)

BRANCH-IV

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL

THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY

CHENNAI, INDIA

APRIL 2015

CERTIFICATE

This is to certify that this dissertation entitled **"TO STUDY THE SEROPREVALENCE OF HEPATITIS E VIRUS INFECTION IN ASYMPTOMATIC PREGNANT MOTHERS ATTENDING ROUTINE ANTENATAL CHECK UP IN A TERTIARY CARE HOSPITAL"**is the bonafide original work done by **Dr. NIVEDHITHA. E,**Post graduate, under my overall supervision and guidance in the department of Microbiology, Stanley Medical College and Hospital, Chennai, in partial fulfilment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

Dr. A.L. MEENAKSHI SUNDARAM, M.D.D.A.,
DEAN
Govt. Stanley Medical College
Chennai-600 001.

Dr. R.SELVI, M.D.,
Professor & H.O.D.
Department of Microbiology
Govt. Stanley Medical College
Chennai-600 001.

DECLARATION

I, solemnly declare that this dissertation**"TO STUDY THE SEROPREVALENCE OF HEPATITIS E VIRUS INFECTION IN ASYMPTOMATIC PREGNANT MOTHERS ATTENDING ROUTINE ANTENATAL CHECK UP IN A TERTIARY CARE HOSPITAL**"is the bonafide work done by me at the Department of Microbiology, Government Stanley Medical College Hospital, Chennai, under the guidance and supervision of **Prof.Dr.R.SELVI, M.D.,** Professor and Head of Department of Microbiology, Government Stanley Medical College, Chennai-600 001.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of degree of M.D. Branch IV Microbiologyexaminations to be held in April 2015.

Place: Chennai. Signature of the candidate

Date :

(Dr. NIVEDHITHA.E)

ACKNOWLEDGEMENT

Firstly, I thank the **ALMIGHTY** for blessing and giving me the health and ability for carrying out this work successfully.

I sincerely acknowledge and wish to express my thanks to Professor **Dr.S.MEENAKSHISUNDHARAM M.D, Dean,**Govt. Stanley Medical College and Hospital, Chennai, for giving me permission to commence this dissertation and to utilize the facilities of this Institution for the study.

I express my warmest respects and profound gratitude to **Dr.R.SELVI, M.D.,** The Professor and Head of The Department of Microbiology, Government Stanley Medical College, Chennai, for her able guidance, constant encouragement and support during the course of the study. My post graduate life has been very rewarding and fulfilling.

I am extremely thankful to **DR. VENKATESHWARAN M.D.,** Professor and Head oftheDepartment of Medical Gastroenterology, for his guidance and support all times.

I would like to thank **DR. KALAIVANI**, professor and HOD, Department of Obstetrics and Gynaecology, RSRM, Government

Stanley medical college and hospital, Chennai, for granting permission to collect blood samples from the antenatal mothers.

I extend my sincere thanks to Assistant Professors Dr. A.V.M. Balaji, M.D., Dr.Shanthi, M.D., Dr.Ponnammal, M.D., Dr.Sheeba, M.D., Dr.Gokulapriya, M.D., of Department of Microbiology for their help, support, interest and valuable advice in needful times.

I express special thanks to my colleague's DR.KAYALVILI.K.Kand DR. S.ARUL SELVAN for their immense help, constant encouragement and unconditional support throughout the study.

I will always cherish the company of my junior PG's and lab technicians of the Department of Microbiology for giving memorable days in my post-graduation study period.

I gratefully acknowledges the help rendered by lab technicians **Mrs. Tamilsuvai and Mrs. Shanas**during sample collection at RSRM, Government Stanley medical college and hospital, Chennai.

I would like to thank **Mr. VinkySingaram**Statistician, during the statistical analysis of the study.

I am indebted to the pregnant mothers from whom blood samples were collected for conducting the study.

I am very profoundly fortunate to be blessed with a wonderful family. I cannot put in words the appreciation for the unwavering support, sacrifice and devotion of my parents.

CONTENTS

TO STUDY THE SEROPREVALENCE OF HEPATITIS E VIRUS INFECTION IN ASYMPTOMATIC PREGNANT MOTHERS ATTENDING ROUTINE ANTENATAL CHECK UP IN A TERTIARY CARE HOSPITAL

OBJECTIVES: HEV infection in pregnant women is more common and fatal in the third trimester. In India there is very less data documented with respect to seroprevalence of HEV in pregnant mothers.The present study was designed to determine the seroprevalence of subclinical HEV infection in asymptomatic pregnant women.

METHODS:200 asymptomatic pregnant womenwith no history of jaundice were studied. Prevalence of anti-HEV antibodies was determined by ELISA and Seminested reverse transcriptase PCR.

RESULTS: The overall seroprevalence of HEV IgM and IgG were 18 (9%)and IgM 12 (6%) in asymptomatic pregnant women. Among them only IgM+ cases were 5 (2.5%), only IgG+ were 11 (5.5%) and both IgM+&IgG+ were 7 (3.5%). No HEV-RNA was detected by RTPCR, which was done for 90 negative cases and all IgM positive cases. This confirms no viremia in the blood and hence no patients were in the window period or active infection. HEV-RNA testing can be considered in patients who are symptomatic for

hepatitis E infection because viremia is the only marker of infection during the acute phase where anti HEV antibodies were not in a detectable levels.

INTERPRETATION & CONCLUSIONS: Present study conducted in Chennai, showed low level of HEV seroprevalence compared to north India. Seroprevalence was significantly associated with overcrowding, type of toilet facility and habit of hand washing with soap after using toilet. As hepatitis E virus spread through faeco-oral contamination, Conservative control efforts at the community level must be undertaken. As HEV is a vaccine preventable infection, it can be considered in the most susceptible and high-risk groups in endemic countries. Early preventive measures if taken, may decrease the maternal and perinatal mortality and morbidity of HEV infection.

INTRODUCTION

Hepatitis is the condition characterized by the presence of inflammatory cells in the liver tissue. It is caused by different aetiological agents and is more commonly used to refer to a group of viral infections that affects liver, such as hepatitis A, B, C, D and E. Hepatitis A and E are transmitted through faeco-oral route, hepatitis B and C are transmitted through infected blood and body fluids and hepatitis D occur exclusively in persons infected with hepatitis B virus.

All water borne epidemic viral hepatitis were thought to be caused by hepatitis A virus. In Delhi there was a suspected outbreak of hepatitis A between 1955 and 1956. Serum samples from those patients were collected and preserved. In 1983 a specific and sensitive assay was applied to these preserved samples and they were found to be negative for specific antibodies against hepatitis A virus[4]. Thus a new clinical entity came into life with signs and symptoms similar to other forms of viral hepatitis.

It was designated as hepatitis E because of its enteric transmission, epidemic and endemic nature that capture the hepatitis E virus[1]. Later it was known as Enterically Transmitted Non A Non B (ENANB) hepatitis or Epidemic Non A Non B[5].

Hepatitis E virus (HEV) causes large outbreaks in endemic areas like India, Central Asia, parts of Africa and Mexico[1]. It is the most important cause of epidemic and sporadic viral borne hepatitis in countries with suboptimal sanitary conditions affecting susceptible population like young aged adults and pregnant women.

Prevalence of Hepatitis E in developing countries ranges from 7.2% to 24.5% when compared to <1% in developed countries[25,32].

Hepatitis E virus causes an acute short lived, self-limiting viral hepatitis typically lasting for 1-4 weeks. It affects both sexes targeting age group between 15 and 40. It exhibits a wide clinical spectrum, fluctuating from asymptomatic infection to fulminant hepatitis. Most cases of pregnancy will not affect the severity of hepatitis unless it is found to be hepatitis E which tends to worsen during the period of pregnancy. It causes fulminant hepatic failure (FHF) in pregnant women particularly those in 2nd and 3rd trimester of pregnancy. There is a high rate of occurrence of disseminated intravascular coagulation (DIC) associated with hepatitis E infection in pregnancy[27]. Attack rate among 1st, 2nd and 3rd trimester were 8.8%, 19.4% and 18.6% respectively. Whereas among non-pregnant women and men it is only 2.1% and 2.8%[2].

37% of acute viral hepatitis during pregnancy is caused by hepatitis E and 81% of them go in for fulminant hepatic failure more commonly during 3rd trimester of pregnancy[20,22]. General mortality rate in hepatitis E is 1 to 2 percentage when compared with hepatitis A which is 0.4 percentage[1].

The case fatality rate among pregnant mothers is 25% [4,22,25] and has been reported to be very high with maximum severity in the third trimester of pregnancy i.e. 44.4%[2] .In fact, the major cause of mortality in epidemics is due to the high rate of fulminant hepatic failure in pregnant women[27]. Besides the high maternal mortality rate, the other dangers of fulminant hepatic failure such as fetal malformation, abortion, still birth and neonatal death are noted among pregnant mothers with hepatitis E infection[4,25].

High rates of vertical transmission of hepatitis E were documented between 33.3% and 50% leading to high fetal mortality[25]. Studies have reported that 78.9% of babies had evidence of vertically transmitted HEV infection[30]. This data points to a relationship between severity of HEV infection in the pregnant women and the fetus.

Most of the cases of hepatitis E remain asymptomatic. The treatment of acute infection is supportive management. In spite of the explosive nature of hepatitis E during pregnancy, there is no

established treatment available for it[31]. Therapeutic termination of pregnancy as approved in disorders like HELLP syndrome have not been completely explored in hepatitis E infection[19]. The diagnosis of hepatitis E infection in individual patients remains challenging. It cannot be clinically distinguished from other forms of acute viral hepatitis. The routine laboratory diagnosis of hepatitis E is based either on serologic tests or nucleic acid amplification techniques[27].

Trials of HEV vaccines are presently continuing in many countries. This consists of a candidate vaccine which is very effective and accepted in China, showing efficacy of >90%. On January 23, 2012 this vaccine has been licensed by State Food and Drug Administration of China for its production and sale. Perhaps it is a major milestone on the road towards protecting susceptible women in a disease endemic country like India.

Hepatitis E is accountable for approximately 9.8% of pregnancy associated deaths. In southern Asia, as many as 10,500 maternal deaths per year could be prevented by using the existing vaccine[24]. With the availability of an efficacious vaccine, we must consider prudent implementation of such an intervention, where appropriate, to avoid a significant proportion of preventable deaths in developing countries.

This study is to assess the seroprevalence of subclinical hepatitis E viral infection in asymptomatic pregnant mothers attending routine antenatal check up in a tertiary care hospital in Chennai. As there is very less data documented with respect to seroprevalence of hepatitis E in pregnant mothers in India. Moreover, it helps in considering vaccination for the pregnant mothers and development of existing vaccine.

Since hepatitis E infection is a significant health problem in the world, there is a need for more public health involvement by provision of clean drinking water, health education of public and easy availability of approved serological assay for early detection of infection.

AIM AND OBJECTIVE

To study the seroprevalence of Hepatitis E virus infection in asymptomatic pregnant mothers attending routine antenatal check up in a tertiary care hospital in Chennai.

REVIEW OF LITERATURE

BACKGROUND

The first description of the clinical features viral hepatitis is recognized by Hippocrates in 460-375 BC. Epidemics of jaundice have been the feature of many army campaigns and have been responsible factor for downfall and triumph of historical wars. In the year 1965, Blumberg et al made a discovery of Australia antigen which consequently led to the uncovering of hepatitis B virus. In 1973, Feinstone et al visualized hepatitis A virus by immune electron microscopy (IEM) in stool extracts of patients with acute hepatitis A virus infection. From post-transfusion patients, Choo et al identified hepatitis C virus and developed serological test for its diagnosis[29].

Until 1980 all water borne epidemic viral hepatitis disease were thought to be caused by hepatitis A. In 1955-56 an epidemic of estimated 29,300 jaundice occurred in Delhi which was reported as a typical cases of hepatitis A disease by Indian Council of Medical Research[13], though diagnostic tests for hepatitis A virus were not existing to confirm or disprove the diagnosis. Following that in November, 1978, in Baramulla District of Kashmir valley a large scale epidemic of jaundice was reported in over 200 villages, covering a

population of 6 lakhs involving icteric disease in 20,000 individuals and with 600 deaths. The disease mainly targeted young adults and documented maximum severity in pregnant women[16]. They recorded notable conclusions[13]:

- The disease was caused by a hepatitis virus
- The epidemic curve compacted lasting for less than 6 weeks
- The spread was through contaminated water
- It was of non-A, non-B type
- It involved young adults with sparing of children
- Typically showed high incidence and severity in pregnant women
- Characteristic histological findings.

In 1980, analysis of the sera collected during these epidemic with a specific and sensitive assay were found to be negative for specific antibodies against hepatitis A [4]. Thus a new clinical entity came into life with signs and symptoms of other forms of viral hepatitis was known as non-A, non-B hepatitis. Indeed, very small number of water born viral hepatitis epidemic in Asia and Africa had been linked to hepatitis A [4].

Later in 1991 One of the biggest waterborne hepatitis E epidemic occurred in Kanpur city, Uttar Pradesh, India. 79,000 clinical cases have been documented. The source of this outbreak was outlined to faecal contamination of drinking water supplied from the river Ganges.

The experimental evidence for the presence of hepatitis E was first reported by Balayanet al[5] in the year 1983. He successfully demonstrated the fecal-oral transmission of hepatitis. The volunteer who had been already infected with hepatitis A infection in the past was given a suspension inoculated with feces specimen collected from the patient named Tashkent resident of Uzbekistan[5].

By 36th day after ingestion of the infected suspension the volunteer developed clinical feature of severe hepatitis. Between 28th to 45th days, fecal sample was collected from the volunteer in which 27 to 30 nm sized virus like particles were detected by immune electron microscopy (IME)[5]. Due to proteolytic degradation smaller viral particles are also excreted in the stool, both sizes of particles were observed in single stool suspension[6].

The volunteer failed to develop serological markers for HAV or HBV but he did develop antibodies to virus like particles which recognized both size of the virus detected from his faeces[5, 6]. Balayanet

al also demonstrated transmission of HEV in cynomolgus monkey with Tashkent's faecal sample and they excreted virus like particles in their faeces[5].

This type of non-A non-B hepatitis was designated as enterically transmitted nonAnonB (ENANB) hepatitis or epidemic nonAnonB. Later it was found to be the major hidden cause for epidemics in many developing countries and also cause sporadic cases of hepatitis.

In those days Immunoelectron microscopy and transmission to primates were the only means of studying ENANB.

CLASSIFICATION

Initially hepatitis E virus (HEV) was grouped within ***Caliciviridae,*** genus ***Calcivirus*** based on their structural characteristics, physiochemical properties, absence of lipid envelope, length of nucleic acid and organization of genome type [4, 6]. Phylogenetic analysis of HEV nucleotide sequence shows it was more closely related to positive strand RNA, with much similarity to Rubella virus, the***Alphavirus*** super family, particularly ***Rubiviruses***[6]. Subsequently, after a long time effort HEV has been reassigned in the new family ***Herpeviridae***genus***Herpevirus***[5] or hepatitis E like viruses in 2004 by Emerson et al[5].

HEPATITIS E VIRUS

Hepatitis E viruses are small icosahedral, symmetrical, non-enveloped, 27-34 nm in diameter with non-segmented positive-sense, single stranded, polyadenylated RNA (at its 3' terminus) and has approximately 7.5 kb genome[4,7].

RNA has 5' region of 27 nucleotide, modified with m^7 G capping and 3' non-coding region of 65 to 74 nucleotides and three partially overlapping forward ORF (Open Reading Frame)[4, 5, 6].

GENOTYPE AND PREVALENCE OF HEPATITIS E HUMAN STRAIN

Genotyping became common in mid-1990's[22]. Hepatitis E strains from different parts of the world were partially or entirely sequenced and based on the phylogenetic analysis it was found that there were four major human HEV strains i.e., I, II, III, IV[5]. Up to 9 genotypes have been proposed based on the limited sequence within the ORF1 region[6]. By Schlauder and Mushahwar, 2001, homology of isolates of the same genotype is not supposed to be less than 81%[7].

Further phylogenetic analysis divided HEV genotype I into 5 subtypes, genotype II into 2 subtypes, genotype III into 10 and finally genotype IV into 7 subtypes[7].

Genotype I – Asia (eg: India, Pakistan, Myanmar, China), North Africa.

Genotype II – Mexico, South Africa.

Genotype III - North and South America, Europe, Asia.

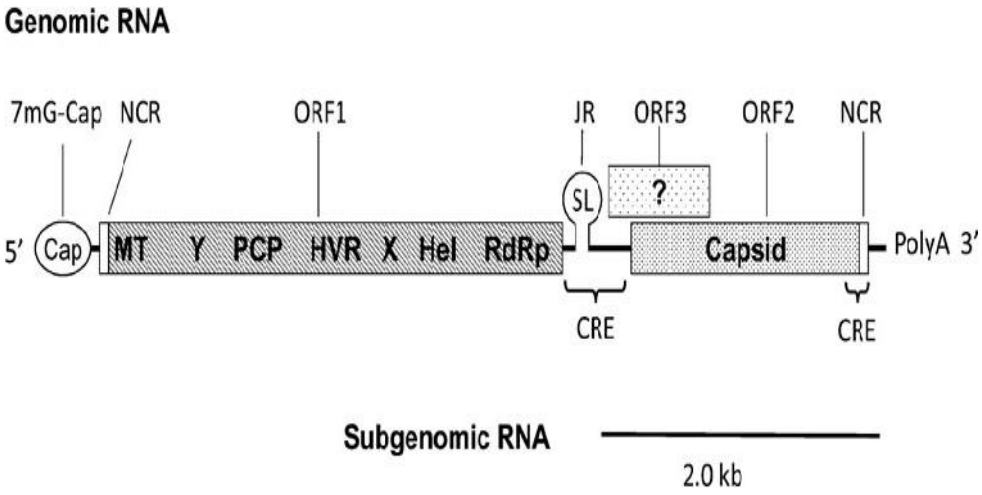
Genotype IV – Asia(China, Taiwan, Vietnam, Japan).

Genotype I and II cause hepatitis outbreak especially in developing countries and Genotype I is the most common subtype in India. Genotype III and IV commonly cause sporadic cases in Asia.

Genotype I contains human hepatitis E strains obtained from endemic regions. Limited sequence strains appears to belong to genotype II. Genotype III and IV have been identified from human and swine sources[6].

MOLECULAR CHARACTERIZATION OF THE VIRUS

Most of the molecular aspects of hepatitis E were gained by in vitro expression of recombinant proteins due to the lack of efficient cell culture system.



OPEN READING FRAME (ORF 1)

ORF1 begins at the 5' terminus of the hepatitis E genome, following long non-coding regions with 27 to 35 nucleiotide[7]. ORF 1 is 5,073 to 5,124 nucleotides long codes for nonstructural proteins (polyproteins with length of 1693 amino acid) that are involved in replication of viral genome and viral protein processing[4, 5,7].

According to Konin et al [7] , ORF 1 is found to have few putative amino acid domains in this gene are as follows:

1. Methytransferase
2. Guanyltransferase
3. Papain-like protease (alpha and rubella viruses)
4. Proline-rich hinge domain (contains hypervariable region and thought to impart flexibility)

5. RNA dependent RNA polymerase
6. NTP binding sequence associated with helicase activity (related to the helicase of superfamily I)[5,7]
7. It has contains two more regions called X and Y domains, whose functions are not determined.

Activity of methyltransferase and guanlytransferase are encoded within the first half of the 5' end and is responsible for capping the hepatitis E viral genome and translation is initiated by capping[5,6,7,28]. Capping enzyme activity was confirmed by the expression of the 1st 979 amino acids in OPF-1[7]. The RNA dependent RNA polymerase is encoded within the 3' half of the ORF1[5,6,7].

However, to date, none of the activities of the domains have been demonstrated.

OPEN READING FRAME 2 (ORF2)

ORF 2 begins at 37nt 3' of the termination coding frame of ORF1. It is composed of 1977 to 1,980 nucleotides [5]. At its 5' end contains three glycosylation signal sequence sites, immediately followed by a region highly basic in charge and rich in arginine. This region is found to encode for the main structural viral capsid proteins.

This is synthesized as a glycosylated 71 kDa, 660 amino acid long precursor protein in the Endoplasmic reticulum. This precursor is then translated directly or through Golgi apparatus to the cell surface. Due to signal sequence it is modified into mature 50-kDa protein, and it is finally processed by C terminus truncation and assembled into the virus particles. It is more usual for the enveloped virus to have extra saccharide than the non-enveloped viruses[5,6,7].

Hepatitis E virus envelope protein form VLPs i.e., Virus like Protein. These particles have been characterized with the help of three-dimensional reconstruction of the structure and Cryo-electron microscopy. In addition to the fresh virions, ORF2 forms 30 small sized icosahedral homodimers (size and modification are not yet determined)[5,6,7].

OPEN READING FRAME 3 (ORF3)

ORF3 is the smallest and last open reading frame. It over laps with ORF1 by 1 nucleotide at its 5' terminal and extends up to 325 to 328 nucleotide in the ORF2 coding frame[5]. It is 366 to 369 nucleotides in length, encodes for pORF protein (123 amino acid) expressed in an intracellular manner, shown to be associated with liver cell cytoskeleton in HIV replication[4, 6].

Near ORF3 amino terminus, has a cysteine rich region which binds with hepatitis E virus RNA and forms a complex with the capsid protein pORF2 after anchoring with pORF3 protein and starts the process of nucleocapsid self-assembly in the virus particles. Other aspects such as assembly and release of viral progeny, some viral protein synthesis, HEV genomic transcription remains unclear.

KEY ANTIGENS[5]

HEV antigens are reactive in immune electron microscopy, enzyme linked immunosorbent assay (ELISA), western blot and immunofluorescentmicroscopy. Major epitopes exist in the carboxy terminal of both ORF2 and ORF3 of HEV strains. ORF3 is more heterogeneous, whereas ORF2 is highly conserved relatively and hence the ORF2 sequence based serological test are broadly reactive and tests containing ORF3 are more strain specific. But exceptions exist[5].

Expressed or synthetic peptides of ORF2 and PRF3 are used in diagnostic tests. Antigens obtained from ORF2 and expressed in insect cells or Escherichia coli have also been used to detect antibodies by enzyme linked immunosorbent assay (ELISA) and Western blot. Truncated proteins expressed from ORF2 in insect cells were better than proteins expressed in Escherichia coli for detecting antibodies against hepatitis E virus. But they fail to detect infections with new

genetic variants of hepatitis E virus. They are also not satisfactorily sensitive for seroepidemiological studies[5].

ORF1 incorporated test are not useful for routine diagnostic purposes, however, important for distinguishing infection-induced and vaccine induced immune response when non replicating hepatitis E vaccines are available commercially[5].

Linear B cell epitope have been recognized in all 3 open reading frames of hepatitis E virus. Epitope mapping of ORF2 and 3 with long manufactured peptides in classic ELISA tests confirms the presence of some, but not all, of the linear epitopes. This suggest that conformational epitopes are vital in the immune response to HEV[5]. In total 26 antigenic epitopes are distributed in the ORF2 gene product[5]. But only 1 neutralization site has identified which is located between 449 and 607 amino acid are conformational[5].

REPLICATION OF HEPATITIS E VIRUS

Little information is known about the stages of replication because hepatitis E virus is not closely related to any other well characterized virus and reports from conventional cell culture are insufficient about its strategy of replication[5, 6].

However, recently, replication of hepatitis E virus have been documented in primary cell cultures of hepatocytes obtained from cynomologus monkeys already infected in vivo. The process of attachment, entry and uncoating of hepatitis E virus is not determined because of limited availability and absence of characterized and permissive cell culture[5, 6].

It is assumed that hepatitis E virus attaches to the receptors on hepatocytes especially in the biliary tract and intestine. From the newly uncoated virus particle, 7.2-kb genome is released. The nonstructural proteins recognizes the capped 5'RNA after presumably translated via cellular mechanisms[5,6] and the required enzymes are provided for the synthesis of both positive and negative strand RNA.

Cellular proteases are found to cleave ORF1 that is sequenced with papain like protease motif, but its functions are not yet clear.

3' end of the viral genome shown to bind with RNA dependent RNA polymerase and ORF 2 binds to the first 76 nucleotide of the 5' region[6].

Positive sense full length genomic RNA and 2 bicistronicsubgenomic mRNA of 3.7 and 2.0kb are transcribed from replicative full length negative strand RNA[5,6].

However, the importance of these subgenomic RNAs in translation of ORF2 and ORF3 protein are not determined. Nothing is known about how these processes are regulated[6].

HEPATITIS E VIRUS TRANSMISSION

Hepatitis E virus is classified as one of the waterborne and foodborne[11]. Most common route of transmission is faeco-oral. Most of the epidemic serologically confirmed for hepatitis E virus have been found to be linked with faecal contamination of drinking water. Some sporadic hepatitis have been associated with consumption of undercooked pork or raw/uncooked shellfish[7]. Consumption of undercooked or raw meat of infected pork, beef, wild boar meat and offal are significantly associated with hepatitis E infection[12]. In Japan, undercooked deer meat and pig liver were found to be a source of acute HEV infection[28].

The spread of hepatitis E virus infection from cases to contact by person to person contact and fomites may play a role, but this is not always common[4]. This may be due to the low amount of intact hepatitis E particles present in a patient's stool[16]. Transmission of hepatitis E virus infection through person to person contact between family members is only one to two percentage compared to fifteen percentage in hepatitis A[7].

Transmission via exposure to infected blood has been reported in endemic regions. Post transfusion hepatitis E infection have also been documented [28]. Transplacental transmission of hepatitis E virus in third trimester of pregnancy is associated high perinatal mortality of the newborns[14].There appears to be relatively higher risk among infants born to mothers with hepatitis E infection upto 33.3% of cases[20]. In a study of 469 pregnant women with hepatitis E infection, found to have vertical transmission up to 100%[19].

Though hepatitis E virus RNA is detected in the colostrum milk, no evidence suggest the spread of infection to offspring[1]. There is no evidence for HEV transmission through sexual route[4]. Hepatitis E infection has also been documented in 3 health care workers who had treated fulminant hepatic failure due to hepatitis E in South Africa[4]. Travelling history to HEV endemic region pose a high risk factor in a number of cases.

Improper treatment of drinking water and substandard sanitation is the major cause for hepatitis E virus outbreaks.

ZOONOTIC POTENTIAL OF HEV

A zoonotic spread of hepatitis E is not left out. Hepatitis E infection is an emerging anthroponosis and zoonosis in the developing

countries. Swine hepatitis E virus has ability to cause infection in pigs and prompted subclinical hepatitis. Experimental inoculation of human hepatitis E though intravenous route in pigs resulted in similar effects[15]. According to Meng et al., 1997, HEV isolated from pigs are in close resemblance to 2 human hepatitis E virus recognized in the United States (US-1 and US-2). Hsieh et al appreciated similar result with different strain of swine hepatitis E virus [7]. Cross species infection has been shown experimentally by Meng et al. Isolates of swine hepatitis E has been shown to communicate disease to chimpanzees and rhesus monkey and the human hepatitis E virus US-2 strain has been shown to be infectious for pigs[7]. Animals like monkeys, cows, rodents, sheep and goats are also susceptible to infection with hepatitis E virus hence they may be a possible source of human reservoir of hepatitis E infection[12].

The recent data indicating presence of markers of hepatitis E infection in multiple animals like pigs, deer, rats, macaques, boars suggest the ubiquitous nature of the virus worldwide. Studies regarding hepatitis E in endemic and non-endemic regions have reported that antibodies to hepatitis E are present in domestic and wild animals. This gives an indication that these animals are in contact with hepatitis E virus or antigenically similar agents[7]. According to Tien et al, 44% of

chicken and 27% of dogs in Vietnam, by Huang et al, 71% of hens in USA, by Okamoto et al, 33% of cats in Japan and also 29% to 62% of cows in endemic regions and 42% to 67% of sheep and goat in non-endemic regions documented antibodies to hepatitis E[7].

This has led to the theory that hepatitis E may represent a zoonotic pathogen, limited to its ecological position until adverse conditions such as floods, ensued faecal contamination of drinking water. Another likelihood is that certain individuals may have extended period of virus shedding and in that way serves as reservoir of HEV infection.

PATHOGENESIS AND PATHOLOGY

PRIMARY HOST TARGET CELLS FOR HEV-HEPATOCYTES[4,5,6,28]

The pathogenesis of Hepatitis E virus infection is not completely understood so far due to the lack of competent cell culture system. Most of the little knowledge about the pathogenesis of hepatitis E virus was obtained from the studies on non-human primates[28]. However, from the clinical picture and its serological events exhibited in some of the typical cases of hepatitis E disease, certain speculative conclusion have been arrived at about its pathogenesis. Ingestion of contaminated

food with infected patient's faeces is the route of primary entry of HEV into the host[5].

It is presumed that hepatitis E virus replication takes place in the intestinal tract. But, the route by which it gains access into the liver is not clearly known but it is supposed to be via the portal venous circulation. It reaches hepatocytes of the liver and starts replication in the cell cytoplasm. The virus first appears in the liver then followed by a phase of viraemia with high concentration of the virus found in bile and is shed in the feces. Transient viraemia and shedding of virus in the faeces occurs for 3-4 weeks. Anti HEV IgM peaks with the symptomatic period and persist for 3 to 6 months and after that they become undetectable.

Anti HEV IgG appears 3 to 4 weeks post inoculation and have been shown to persist for 2 to 13 years. During convalescent period IgG disappears within 6 to 12 months period[1]. HEV RNA are detected in the biliary duct, on the luminal aspect of the epithelial cell surface[7] using in situ hybridisation. Negative –strand viral replicative RNA was detected the small intestine, colon, spleen, lymph nodes in swine HEV inoculated pigs[8]. The template RNA was also detected in liver, spleen, stomach, small intestine, colon, tonsils, kidney and salivary glands[9].

Hepatitis E virus antigens are expressed in the hepatocyte

cytoplasm, faeces and bile. Antigens appears one week post infection indicating viral replication. HEV can be detected in the stool a week prior to the clinical symptoms.

It has been established that the liver injury is caused by the immune response produced by the host to the invading hepatitis E and not depends directly on the replication of the virus in the liver tissue. Viremia and faecal shedding is followed by the onset of ALT elevation and observable histopathological changes in the liver usually correspond to the detectable levels of antibodies to hepatitis E in the serum and decreased levels of hepatitis E virus antigens in the hepatocytes of the liver.

During epidemic outbreaks of hepatitis E disease, examination of the infected patient’s liver shows two general types of morphological changes. A characteristic cholestatic pattern, glandular modification of the parenchymal cells and notable bile stasis within the canaliculi of the liver called cholestatic form.

Immune mediated reaction of the host results in the infiltration of the liver cells with polymorphonuclear leucocytes and lymphocytes which may be the cause for the liver injury and they have been found to have suppressor or cytotoxic immunophenotype.

A second type of histopathological pattern observed is similar to the other forms of acute viral hepatitis which includes acidophilic bodies' formation and ballooning degeneration of the hepatocytes or confluent hepatocyte necrosis in the liver [4,6].

PREGNANCY AND HEV

Pregnant women, particularly those in 2nd and 3rd trimester of pregnancy are more frequently affected during hepatitis E outbreaks. They have a worse outcome than other form of viral hepatitis.

It was proposed that severe fetal infections and fetal death may produce toxins that overload circulation which causes severe maternal disease[27]. During pregnancy there is increased sensitivity to hepatitis E virus mediated endotoxins[1] called Schwartzman-like phenomenon. Hepatitis E virus infection are fatal due to hepatic and renal failure as they precipitate pregnancy associated eclampsia with disseminated intravascular coagulation[28].

Following are the possible host factors that favor's fulminant hepatic failure to occur in hepatitis E infected pregnant women's:

Immunological changes

Hormonal changes during pregnancy

Genetic and environmental factors

IMMUNOLOGICAL CHANGES DURING PREGNANCY[28]

During the early weeks of gestation up to 20th weeks, T cells are significantly reduced. It is a protective modulation of cell mediated immunity (CMI) to sustain the highly antigenic fetus and the placenta during pregnancy. Placenta are resistant to cell mediated damage as they do not express MHC.

They express a unique HLA-G molecule which inactivates Natural Killer cells by binding to its receptors CD16 and CD56. It also produces indoleamine 2,3dioxygenase enzyme, inactivate and deplete the aminoacid tryptophan, which supports T cell function and hence suppresses CMI. Increase in cytokines such as TGF-β, IL-4 and IL-10 inhibits cell mediated immunity.

HORMONAL FACTORS IN PREGNANCY

Increased incidence of FHF may be associated with hormonal changes. As the period of gestation progresses the hormones like estrogen, progesterone and human chorionic gonadotropin also increases. Estrogen causes shrinkage of thymus and deplete the number of CD4 and CD8 cells. Progesterone blocks T cell development i.e. they inhibit Th1 cell and promote Th2 cells by producing involution of thymus more effectively than estrogen. It was also shown that there is

increased level of T helper2 cytokines assuming this may cause liver injury and have a role in increased severity of HEV infection in pregnancy[25].

PROBABLE MECHANISM OF FATAL FULMINANT HEPATIC FAILURE IN PREGNANCY[19]

In addition to the above mentioned hormones, high levels of steroid hormones are also appreciated during pregnancy. Through NF-κB steroids mediate lymphocyte apoptosis and this physiological down regulation helps to retain the fetus. In studies it was shown that the activity of p65 component of NF-κB was decreased in peripheral blood mononuclear cells as well as liver biopsy of pregnant women died of fulminant hepatic failure. It was established that absence of p65 is responsible for liver damage in fulminant hepatic failure associated in hepatitis E infected mothers.

CLINICAL MANIFESTATION

Incubation period ranges from 2 to 8 wks for the clinical manifestation of the disease. Wide spectrum of clinical manifestation have been observed, from self-limiting, subclinical, acute hepatitis to fulminant hepatitis in case of pregnant mothers but never proceeds to chronicity.

It is most commonly seen in the age group of 15 to 45. It is mostly asymptomatic in children. 1% mortality of general population is due to FHF.

SIGNS AND SYMPTOMS

PRE-ICTERIC OR FIRST PHASE

Influenza like symptoms

Abdominal pain

Tenderness

Nausea

Vomiting

ICTERIC OR SECOND PHASE

Jaundice

Dark urine

Viremia

Elevated liver enzymes

Antibody seroconversion

Clearing of the viruses

First phase or pre-icteric phase lasts for 1 to 10 days followed by second or icteric phase persist for 15 to 40 days.

OUTCOME OF HEV

Typically hepatitis E virus infection is self-limiting without progression to the chronic illness. However hepatitis E infection is more severe than hepatitis A. It causes fulminant hepatic failure in pregnant women's and can be fatal.

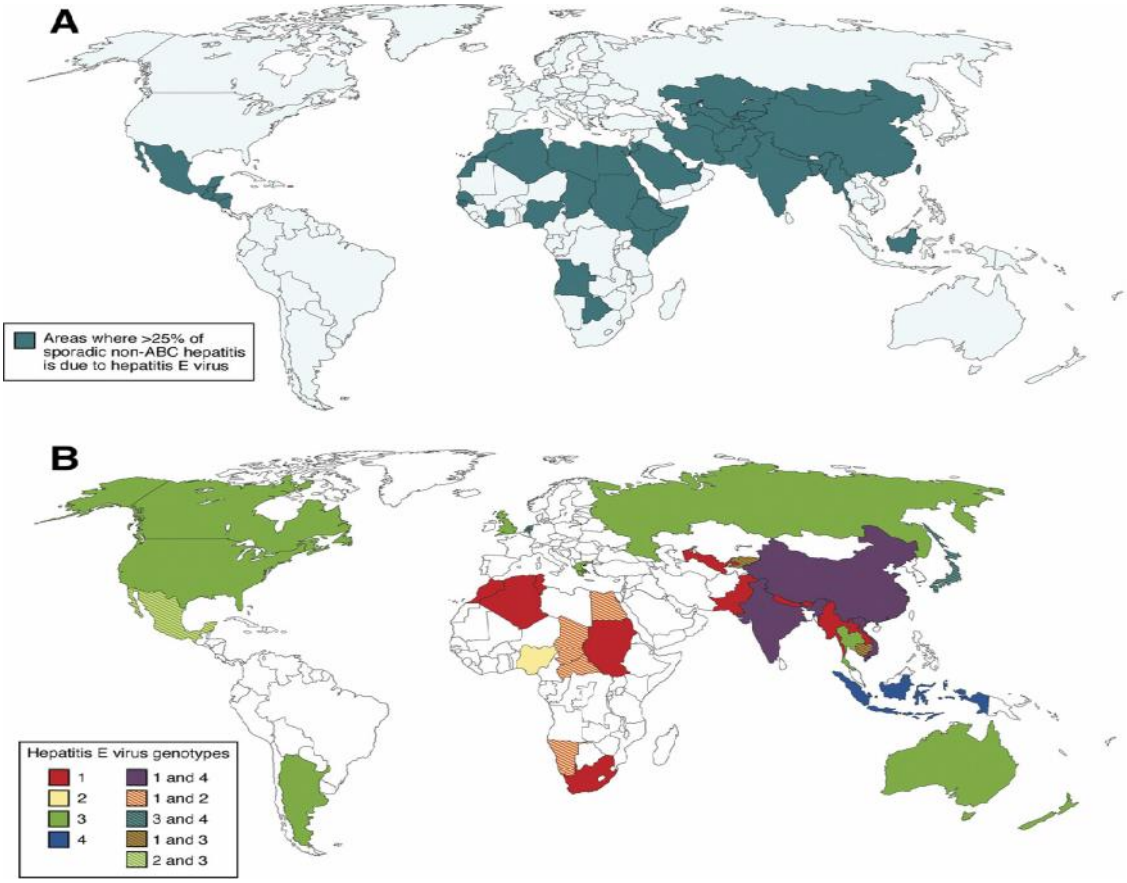
Fulminant form of hepatitis E infection occurs more frequently in the third trimester of pregnancy with a mortality rate of 25%.

However, on the basis of clinical presentation hepatitis E cannot be differentiated from other forms of viral hepatitis. In fact, not all hepatitis E infections are clinically apparent.

Increased frequency of abortion, neonatal deaths and still births are noted among pregnant mothers with hepatitis E infection[4]

Other complications are prolonged cholestatic hepatitis, acute HEV superinfection in patients with cirrhosis, co-infection with other hepatotropic viruses and autochthonous hepatitis E virus in developed countries

SEROEPIDEMIOLOGY



Hepatitis E cause waterborne epidemic disease with peak clinical attack rate in young adults and high rate of fulminant hepatitis in pregnancy. The earliest recorded outbreak of HEV occurred in the year 1955 in Delhi, India, following heavy flooding of the river Yamuna. Thereafter, many number of outbreaks and sporadic cases have been documented in a wide variety of developing and tropical regions, together with, India, Nepal, Pakistan, Myanmar, Indonesia, china, the central Asian region of the former Soviet Union, Ethiopia, Egypt, Algeria, Jordan, the Ivory Coast, Sudan, Chad, Somalia, Ethiopia, and Mexico[4,5].

Sporadically occurring hepatitis in many of the regions have been serologically confirmed as hepatitis E virus infection. In fact, hepatitis E virus is one of the most common etiology of sporadic hepatitis in the endemic regions.

Salient feature of hepatitis E is that it has age specific clinical attack rate with its peak among young adults ranging from 15 to 40 years of age in developing countries[1]. Survey of sera collected from a hepatitis E endemic area of India over a period of ten years, documented that most hepatitis A infection occurred before five years of age, whereas, hepatitis E virus infection occurred after sixteen years of age. However, all age groups are affected with male preponderance and mostly adults develop clinical evidence of hepatitis than children during epidemics[2,3].

In most hepatitis E endemic areas, antibodies have been identified in 5% of children in the age group less than ten years when compared to 10% to 40% in young adults >25 years of age[4]. Recent study from India have reported >60% of children <5 years of age have antibodies against hepatitis E virus[4].

By Nargis et al, seroprevalence of anti-hepatitis E IgG in New Delhi, India is 33.67% in asymptomatic healthy mothers[2]. Whereas in Spain and Turkey prevalence it is 0.6-2 and 12.6 percent respectively[2].

Arankalle et al, seroprevalence of IgG in general population is 23.62%[2]. By Khuroo et al, it is 5% in asymptomatic in healthy children[2].

By Radhakrishnan et al, among 361 cases of acute viral hepatitis 17.3%(66) were positive for IgM[29]. By Singh et al 40% of pregnant women were positive for IgM anti HEV antibody[29]. By Bista et al, out of 19 jaundiced pregnant women 16 (84.2%) were positive for anti HEV IgM antibody[29].

Relatively high seroprevalence rate were reported in developed countries like japan (4%-7%) and U.S (15%- 20% in blood donors)[28]. The urban sewage samples from non-endemic areas like Spain, Greece, France, Sweden, and U.S tested positive for hepatitis E virus infection[28], signifying that the healthy population of non-endemic areas may also be exposed to other types of animal HEV strains that do not develop any clinical manifestations.

Development of sensitive serological assay has permitted for complete analysis of HEV seroprevalence and its distribution worldwide. Unpredictably, the prevalence of anti HEV IgG in noted endemic regions has been much lower than anticipated and the occurrence of such anti HEV in non-endemic regions has been in much higher levels.

Molecular methodology to the epidemiology of hepatitis E virus hold some promise. The moderately low level of hepatitis E in blood and faeces restricts the practicality of hybridization without amplification. RT-PCR has been useful for checking the outcomes of serological test and for assessing the duration of infectivity of the cases. Limitation to molecular epidemiological studies are due to fairly short duration of faecal shedding and viremia. Its main significance is identification of different types of hepatitis E virus genotypes and their association to specific epidemics and environmental locations. Furthermore, RT-PCR has been used to identify hepatitis E in sewage and contaminated water.

RISK GROUP IN THE POPULATION[7,16]

1.People living in areas where community outbreaks of hepatitis E infection occurs

2. People living in overcrowded refugee camps following disastrous events

3. People travelling to hepatitis E endemic areas

4. People with chronic liver disease

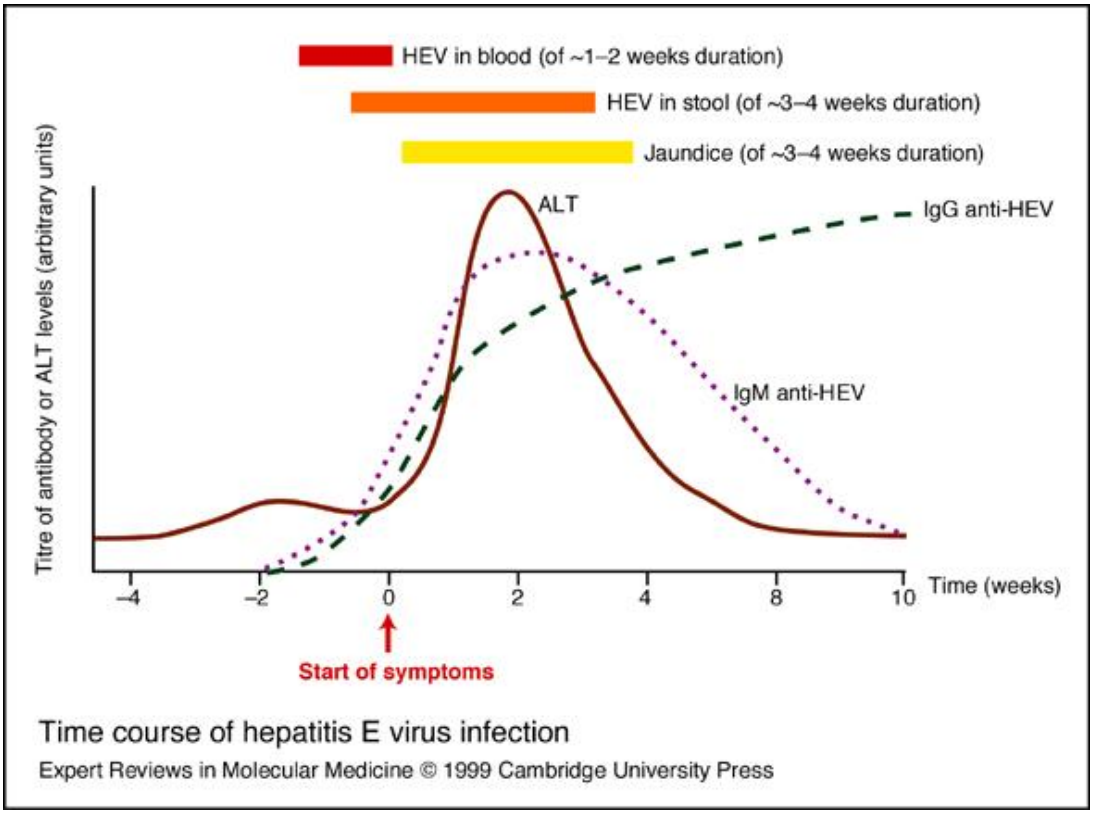
5. People handling pigs, cows, sheep, goat and non-human primates which may be infected with hepatitis E virus.

6. Travellers to the endemic areas

DIAGNOSIS

Hepatitis E should be should be suspected in endemic areas during outbreaks of water-borne hepatitis in people living in sub optimal sanitary conditions. In developed nations hepatitis E should be suspected in patients presenting with signs and symptoms of hepatitis with history recently returned from endemic areas. Similar to the other forms of viral hepatitis, serological tests play an important role in establishing a definitive diagnosis of hepatitis E.

Specific test for anti HEV IgM and IgG antibodies are available. IgM is the acute phase marker and IgG determines the exposure to HEV. Detection of IgM is up to 90% during acute infection if the suspected patient's serum samples are collected between 1 and 4 weeks after the onset of symptoms. During the first 4 weeks of infection after the onset on disease, IgM reaches peak titer of 1:1000 to 1:10000 and disappears within 3 months in 50% of the patients[5]. Between 2 and 4 weeks, IgG peaks in titer of 1:1000 to 1:100000 and thereafter diminishes rapidly relatively[5]. According to Clayson et al., in the infected people IgG antibodies against HEV persist for more than 14 years in 47% of the patients[7].



Time course of hepatitis E virus infection

IMMUNOLOGICAL DIAGNOSIS

ENZYME IMMUNO ASSAY (EIA)[7]

IMMUNOFLUORESCENCE MICROSCOPY (IFE)

WESTERN BLOT

EIA is a highly sensitive and inexpensive and a practical method for detection of antibodies against hepatitis E virus.

IgM- acute phase marker

IgG- measure exposure to hepatitis E

Specific antibodies can be detected in either blood or serum. Following are the antigenic domains found in the ORFs proteins.

1. ORF1 – 12 antigenic domains
2. ORF2 – 6 antigenic domains
3. ORF3 – 3 antigenic domains

Complete ORF3 and large segment of ORF2 or ORF3 C end domain and recombinant proteins originating from the ORF2 are used for detection of IgM and IgG antibodies of HEV. In the convalescent stage of the disease course, ‘capsid-like’ or large ORF2 particles are more effective in detection of anti HEV.

Synthetic peptide antigens are used to confirm enzyme immune assay results and to exclude nonspecific reactions. Their use increase the specificity of the reaction and helps to determine the genotype of hepatitis E. Disadvantage of this peptide antigens is that it has low sensitivity and not reliable in detection of antibodies in the convalescent period[7].

Four short recombinant proteins derived from 3’ end of ORF-2 with 42 amino acid and ORF-3 with 33 amino acid of Burmese (genotype –I) and Mexican (genotype –II) are used by two Genelab EIA. 2 recombinant proteins obtained from the complete ORF3 with 123

amino acid of the Burmese genotype I strain by Abbott –EIA[7]. Sensitivity, specificity, positive predictive value and negative predictive values of all three combination of EIA's are 100%, 99.5%, 75% and 100% respectively[7].

According to Mast et al, 12 different enzyme immune assay showed a concordance from 41 to 94 percent in blood donors and 0 to 89% among reactive sera with mean 68 and 32 percent respectively[7].

During acute HEV infection inflammation of liver or damage to liver tissues can be assessed by elevated liver enzymes like glutamyltranspeptidase (GGT) alanine aminotransferase (ALT)

IMMUNOFLUORESCENCE MICROSCOPY (IFE)

This is a semi-quantitative test measures the concentration of anti HEV antibodies against hepatitis E virus. Fluorescein–conjugated anti-HEV IgG to hepatitis E antigen in the liver tissue is blocked by anti HEV antibodies.

IFE is expensive and laborious and hence not useful for routine diagnosis.

MOLECULAR DETECTION OF HEV

IMMUNE ELECTRON MICROSCOPY

NUCLEIC ACID AMPLIFICATION TEST

VIRAL ISOLATION

IMMUNE ELECTRON MICROSCOPY (IEM)[17]

Balayan et al., 1983 used IEM to detect virus like particles in the clinical specimens. Antibodies to HEV derived from acute phase or convalescent phase sera, precipitate the HEV particles. By rating the antibody coating, concentration of the anti HEV antibody can be determined semi-quantitatively.

For routine analysis, immune electron microscope (IEM) is not suitable as its sensitivity and specificity of the assay is insufficient. Moreover clinical samples contains less number of virus like particles to be detected by IEM.

Molecular methods in diagnosis of hepatitis E have replaced Immune electron microscopy as it is positive in only 10% of cases[5].

NUCLEIC ACID AMPLIFICATION TEST

They can be used to detect HEV RNA either in serum/blood or stool. These tests are of importance during the window

period as the specific antibodies to HEV are not mounted to the detectable levels, hence the serological test are found to be negative[3].

Nested RT PCR and real time RT PCR are nucleic acid based techniques is the method of choice for the specific detection of HEV RNA in blood and faeces during the acute phase of infection. This method is very advantageous in detecting divergent hepatitis E virus strains in non-endemic countries where some assay fail to detect serological response to hepatitis E. But the sensitivity of the test depends on a proper match between the hepatitis E virus strain and the PCR primers.

Conventional RT-PCR detects viral RNA, not only from sera and faeces in humans and also detects from clinical samples like bone marrow, plasma, serum, salivary gland, mesenteric lymph nodes, inguinal lymph nodes, kidney, urine, liver and bile of domestic pigs and other animals like wild boar, sika deer, mongoose, chickens and also in contaminated water[7].

VIRAL ISOLATION[17]

For viral characterization and diagnosis, establishment of a practical cell culture that allow the multiplication of hepatitis E in vitro is very critical. The human lung diploid cell culture system (2BS,

PLC/PRF/5, A549,HepG2) and primary cell culture system from non-human primates like chimpanzees, African green monkeys, cynomolgus macaques and tamarins have been reported[7,17].

But these cell culture medium cannot be used for virological and biophysical studies of hepatitis E because they do not provide high-tire of HEV in the culture medium and have reproducibility.

Toshinori Tanaka et al used Fecal suspension with 2.0×10^{-7} of HEV copies ml^{-1} were used successfully in developing a cell culture system with an HEV titre of up to 10^8 in PLC/PRF/5 cells [17].

TREATMENT

Treatment of hepatitis E virus is supportive and involves bed rest, adequate hydration and electrolyte repletion. Almost all of them able to clear the virus spontaneously. There is no specific therapy available in altering the course of acute hepatitis E infection. Antivirals have not been effectively established for the treatment of hepatitis E infection. In acute severe form of infection, patients are treated with ribavirin for 21 days. Pilot studies in cell culture suggest that interferon alpha and ribavirin may inhibit hepatitis E virus[1].Though ribavirin is contraindicated in pregnancy for its teratogenicity, the risk of untreated hepatitis E infected mother and fetus are essentially high.

PREVENTION OF HEPATITIS E VIRUS

Following are the most important means of prevention

- Good personal hygiene
- High quality public water supplies
- Proper disposal of sanitary waste and
- General food safety

RECOMMENDATION[16]:

1. Disease surveillance and outbreak detection

    a. Enhance hepatitis E diagnosis and reporting in all acute hepatitis cases particularly those that test negative for hepatitis A and hepatitis B virus to enable epidemiological investigation and out breaks

    b. Use of molecular methods to determine the different genotype prevalence in an area.

2. Food and water safety

    a. Increased awareness among food handlers

    b. Food surveillance programme for hepatitis E

3. Public health education

a. Sea foods like shell fish, pork and pig offal should be cooked completely before consumption

b. To take appropriate measures to prevent hepatitis E infection when travelling to an endemic areas

4. Local study

To monitor hepatitis E prevalence and epidemiological changes

SWINE HEV

For the first time in the year 1997 by Meng et al, HEV from the clinical samples of pigs in USA was identified and demonstrated. Subsequently, other countries from all over the world with high production of pork, hepatitis E virus strains have been detected. These hepatitis E genotypes showed their association with genotype III and genotype IV. Swine HEV strain is almost homologous with the human hepatitis E from the same geographic location(Hsieh et al, Pina et al, Wang et al, Huang et al, Yazaki et al)[7,]. The swine hepatitis E virus shared more than 97% amino acid identity with human hepatitis E genotype III[16]. Isolates of hepatitis E virus strains from Thailand and Mexico has been classified as genotype III where human isolates of

hepatitis E has been categorized as genotype I and II. In India human HEV strains are designated as genotype I and swine as genotype IV[7].

AVIAN HEV

In U.S., chickens suffered with Hepatitis-splenomegaly syndrome from whom the novel HEV strain, the avian HEV was isolated from the bile samples by Haqshenaset al[7]. Comparison of avian HEV and BSLV (big liver and spleen disease virus) revealed resemblance of about 80%. Sequenced genome segment of isolates of avian HEV showed 50 to 60 percent similarity with human and swine HEV strains[7]. Variation in the position of ORF was observed, ORF 3 does not shown overlapping with ORF-1 unlike classical hepatitis E viral genome. According to Wang et al, and Haqshenas et al, it is not clear that whether isolates of avian HEV has different V of hepatitis E or it is another member of Herpevirus genus[7]. Later, phylogenetic analysis and sequence comparison of avian HEV discovered that it is most divergent of the hepatitis E virus strains and had 50% sequence identity[28]. Newly, another new avirulent strain of avian HEV was isolated from seemingly healthy chickens. It is assumed that the 2 avian HEV strains fit into presumed genotype V[28].

ANIMAL EXPERIMENTS

Several species of monkey and chimpanzees are susceptible to hepatitis E virus infection. Most of our understanding of the pathogenesis of hepatitis E infection is derived from the reliable non-human experimental animal models like rhesus, cynomolgus macaques, owl monkeys and tamarins.

Incubation period was 21 days after intravenous inoculation of Hepatitis E virus into cynamolgus macaques. During the initial highly replicative cycle, expression of HEV Ag appears approximately 7^{th} day post infection[4,6]. During 2 or 3 week after inoculation, HEV Ag has been detected in feces, bile and hepatocyte cytoplasm due to excretion of HEV into bile.

Antigenic expression of hepatitis E covers approximately 70%to90% of the hepatocytes and the quantity of viral antigen declines with the onset of raise in liver enzymes and there was fecal excretion of virus and viremia in the blood. Detectable antibodies to hepatitis E virus appears just before the elevation of ALT(alanine aminotransferase) and coincides with resolution of faecal virus shedding, viremia in the blood and reduction in the viral antigen in the liver and correspond to the presence of histopathogical changes in the liver. In the last decade, a growing trend of hepatitis E notification has

been observed. From 2001 to 2010, the yearly notification of hepatitis E infection in the past decade has ranged from 26 to 117. In fact, hepatitis E turn out to be the most common cause of viral hepatitis reported in 2010. Hepatitis E accounts for 44.3% of all viral hepatitis cases, followed by 27.7% of viral hepatitis B and 24.2% of viral hepatitis A

India is endemic for hepatitis E infection and it is the most common cause of viral hepatitis in pregnancy with high mortality during 3rd trimester following fulminant hepatic failure which may be associated with the hormonal changes during the course of pregnancy[22].Hepatitis E virus gives a large global burden of sporadic and epidemic hepatitis.

MATERIALS AND METHODS

TYPE OF STUDY:

Prospective study.

STUDY PLACE:

This study was done in the Department of Microbiology, Stanley Medical College and Hospital in association with Department of Obstetrics and Gynecology, RSRM and Medical Gastroenterology, Stanley Medical College and Hospital, Chennai.

PERIOD OF STUDY:

Jan 2014 – sept 2014

SAMPLE SIZE:

200

INCLUSION CRITERIA

All the asymptomatic pregnant mothers who were attending the antenatal OPD and who were willing to provide written informed consent were recruited for the study.

EXCLUSION CRITERIA

Antenatal mother with signs & symptoms of hepatitis.

PATIENT SELECTION:

The study was conducted among the asymptomatic pregnant mothers attending routine antenatal check-up in the Department of Obstetrics and Gynaecology, RSRM and Medical Gastroenterology, at Government Stanley Medical College and Hospital, Chennai for a period of 9 months from Jan 2014 to Sept 2014.

Population group were from in and around Chennai.

The study was explained in detail to the pregnant mothers in their local language and informed consent were obtained.

All asymptomatic antenatal mothers who were willing to give informed consent irrespective of their gestational age were enrolled in the study.

No samples were repeated from the same patients.

200 asymptomatic pregnant mothers were selected for this study.

Blood samples were collected and serum was separated from them to detect the presence of IgM and IgG antibodies to HEV and to detect viral RNA by PCR.

ETHICAL CONSIDERATION:

Ethical and research clearance was obtained from the ethical committee Stanley Medical College and Hospital. Permission to

conduct the study was sought from the respective hospital authorities. Informed consent was obtained in the local language of the patient before enrolment into the study.

Statistical analysis:

The collected data was analysed with SPSS 16.0 version. To describe about the data descriptive statistics frequency analysis, percentage analysis, cross tabulation were used for categorical variables and the mean and S.D were used. To find the significance in categorical data Chi-Square test was used. In all the above statistical tools the probability value .05 was considered as significant level.

DATA COLLECTION:

Details were obtained directly from the patients with the help of a questionnaire which dealt with information regarding sociodemographic data such as age, residential address, educational status, profession, socioeconomic status, source of drinking water and type of toilet facility to analyse various factors that contribute for the prevalence of HEV infection in pregnant mothers in the particular geographic area.

SAMPLE COLLECTION:

Under aseptic precaution around 5 ml of blood sample was collected from each patients by venipuncture at the cubital fossa, by using 23G needle. Blood was dispensed into a sterile test tube without anticoagulant. Samples were transported immediately to the Microbiology lab. The blood was centrifuged at 2500 rpm and the serum was separated. Samples were duplicated and stored in a storage vials containing 50µl of EDTA at -80°C deep freezer.

One set of serum samples were used for doing indirect ELISA (Enzyme Linked Immunosorbent Assay) to detect both IgG and IgM antibodies against Hepatitis E virus. Other set of samples were used for HEV-RNA detection using Seminested Reverse Transcriptase PCR (HELINI biomolecules).

ELISA (Enzyme Linked Immunosorbant Assay)

ELISA was done with a commercial kit

1. DSI-EIA-ANTI-HEV-IgM-KIT
2. DSI-EIA-ANTI-HEV-IgG-KIT

This test was intended for the screening of serum IgM and IgG antibodies against HEV. The test was performed according to the instructions provided in the kit literature.

PRINCIPLE OF THE TEST:

TYPE OF ELISA: INDIRECT ELISA

Diluted test serum samples when added to the HEV antigens coated strips, if specific antibodies to the antigen are present in the serum, they get bound to each other in the wells during the incubation period. Unbound antibodies were removed during washing. When freshly prepared enzyme conjugate was added, it specifically binds to the antigen antibody complex that had formed during the first incubation. Unbound conjugate were removed during washing. To this a chromogen substrate was added. The peroxidase enzyme present in substrate catalyse the reaction that consumes peroxide and turned the chromogen from clear to blue. Addition of stop solution end the reaction and turned the blue colour to a bright yellow colour. The reaction was read with an ELISA reader.

MATERIALS AND EQUIPMENTS REQUIRED

1. Purified water
2. Pipettes to measure and dispense 10, 50, 90, 100
3. Pipette tips
4. Incubator at 37.0±1.0 C
5. Automated microplate washer

6. Microplate reader equipped with 450nm
7. Disposable gloves

REAGENTS

1. 96 microtitre wells coated with HEV antigens
2. Wash buffer
3. Sample diluent
4. Enzyme conjugate and diluent
5. TMB substrate and substrate buffer
6. Stop solution
7. Positive control (inactivated)
8. Negative control(inactivated)

HANDLING OF SPECIMENS

Plasma stored at -80°C was thawed for few minutes at 40°C to avoid fibrin precipitation.

AUTOMATIC MICROPLATE WASHER:

Automatic ELISA plate washer was set up appropriately i.e. 380-400µl working washing solution into each wells and soak time at least 40 seconds and aspirate.

PREPARATION OF THE REAGENTS

HEV –Ag COATED STRIPS

The foil bag was opened and the strips were removed and placed in the microtitre plate. The strips were numbered and the wells were assigned with the respective samples with the help of a data sheet. Before initiating the assay all the strips were washed 2 times with the automatic microplate washer, as instructed in the manual.

PREPARATION OF WASH BUFFFER

To 50ml of washing buffer (concentrated 25 fold) 1200ml of distilled water (1:25 ratio) was added and mixed thoroughly according to the manual.

PREPARATION OF THE WORKING CONJUGATE

Working conjugate was prepared just before the test procedure. To 0.65ml of conjugate (concentrated 21 fold) 13ml of conjugate diluent (1:20 ratio) was added and thoroughly mixed until diluted. Intensive mixing was avoided.

PREPARATION OF THE SUBSTRATE MIXTURE

The substrate mixture was prepared just before use. To 0.65ml of TMB (concentrated 21 fold) 13ml of substrate buffer (1:20 ratio)

was added and mixed thoroughly until diluted. This step was done after darkening the room as it was sensitive to light.

DS-EIA-ANTI-M ASSAY PROCEDURE:

BEFORE ASSAY

The coated strips was washed with working washing solution 2 times before the procedure. The microtitre plate was tapped over tissue paper and was made sure thatfluid or bubbles were not left inside the wells as they may adversely affect the assay precision. Data sheet are made ready for the controls and for the samples.

1. 100µl of Positive control was added into one well and Negative control was added in 2 wells.
2. 90µl of sample diluent and 10µl of test serum were added in the rest of the wells.
3. The plate was covered by a lid protective film.
4. The plate was incubate for 30 minutes at 37±1°C in the incubator.
5. The contents was removed by washing the plate for 4 times.
6. 100µl of freshly prepared working solution of conjugate was then added into each wells.

7. The plate was covered by a lid protective film.
8. The plate was incubate for 30 minutes at 37±1°C in the incubator.
9. The contents was removed by washing the plate for 4 times.
10. 100µl of freshly prepared substrate mixture was added into each wells.
11. The plate was kept in a dark place for 20 minutes at 18-24°C
12. 150µl of stopping reagent was added to all the reacting wells.
13. Optical density at 450/620 nm using microplate reader was used to read the result immediately after adding stop solution.

Validation of the test was confirmed by the following:

Positive control > 0.6 and Negative control < 0.2

CUT – OFF = AVERAGE OF NEGATIVE CONTROL + 0.200 (coefficient defined by manufacturer during statistical processing for each lot)

INTERPRETATION OF RESULTS

Sample was considered NEGATIVE if OD < CUT – OFF

Sample was considered POSITIVE if OD ≥ CUT – OFF

PREPARATION OF THE REAGENTS FOR IgG ANTI HEV

HEV –Ag COATED STRIPS

The foil bag was opened and the strips were removed and placed in the microtitre plate. The strips were numbered and the wells were assigned with the respective samples with the help of a data sheet. Before initiating the assay all the strips were washed 2 times with the automatic microplate washer, as instructed in the manual.

PREPARATION OF WASH BUFFFER

To 50ml of washing buffer (concentrated 25 fold), 1200ml of distilled water (1:25 ratio), was added and mixed thoroughly according to the manual.

PREPARATION OF THE WORKING CONJUGATE

Working conjugate was prepared just before the test procedure. To 0.65ml of conjugate (concentrated 21 fold), 13ml of conjugate diluent (1:20 ratio) was added and thoroughly mixed until diluted avoiding foaming. Intensive mixing was avoided.

PREPARATION OF THE SUBSTRATE MIXTURE

The substrate mixture was prepared just before use. To 0.65ml of TMB (concentrated 21 fold), 13ml of substrate buffer (1:20 ratio)

was added and mixed thoroughly until diluted. This step was done after darkening the room as it was sensitive to light.

DS-EIA-ANTI-M ASSAY PROCEDURE:

BEFORE ASSAY

The coated strips was washed with working washing solution 2 times before the procedure. The microtitre plate was tapped over tissue paper and was made sure that no fluid or bubbles are left inside the wells as they may adversely affect the assay precision. Data sheet are made ready for the controls and for the samples.

1. 100µl of Positive control was added into one well and Negative control was added in 2 wells.
2. 90µl of sample diluent and 10µl of test serum were added in the rest of the wells.
3. The plate was covered by a lid protective film.
4. The plate was incubate for 30 minutes at 37±1°C in the incubator.
5. The contents was removed by washing the plate for 4 times.
6. 100µl of freshly prepared working solution of conjugate was then added into each wells.

7. The plate was covered by a lid protective film.
8. The plate was incubate for 30 minutes at 37±1°C in the incubator.
9. The contents was removed by washing the plate for 4 times.
10. 100µl of freshly prepared substrate mixture was added into each wells.
11. The plate was kept in a dark place for 20 minutes at 18-24°C
12. 150µl of stopping reagent was added to all the reacting wells.
13. Optical density at 450/620 nm using microplate reader was used to read the result immediately after adding stop solution.

Validation of the test was confirmed by the following:

Positive control > 0.6 and Negative control < 0.2

CUT – OFF = AVERAGE OF NEGATIVE CONTROL + 0.200 (coefficient defined by manufacturer during statistical processing for each lot)

INTERPRETATION OF RESULTS

Sample was considered NEGATIVE if OD < CUT – OFF

Sample was considered POSITIVE if OD ≥ CUT – OFF

NUCLEIC ACID AMPLIFICATION TEST BY REVERSE TRANSCRIPTASE PCR

RNA EXTRACTION:

PRINCIPLE:

Cells are lysed during a short incubation with chaotropic salt, which immediately inactivate all nucleases. Cellular nucleic acids bind selectively to special glass fibers pre-packed in the purification filter tubes. Bound nucleic acids are purified in a series of rapid ‘wash and spin’ steps to remove contaminating cellular components.

A special inhibitor removal buffer has been included which removes inhibitors from the preparation. Finally elution buffer releases the nucleic acids from the glass fibre. This simple method eliminates the need for the organic solvent extractions and nucleic acid precipitation, allowing for rapid purification of many samples simultaneously.

MATERIALS REQUIRED

1. Micro pipette variable volume 0.5-10µl , 10-100µl and 100-1000µl
2. Sterile pipette tips with aerosol barrier 0.5-10µl, 2-20µl, 10-100µl and 100-1000µl

3. Disposable powder free gloves
4. Pure fast spin column
5. Vortex mixer
6. water bath
7. Centrifuge with rotor for 1.5 ml reaction tube
8. 1.5ml/ 2ml centrifuge tubes

SAMPLE: serum samples

Before use all samples and reagents were thawed completely, mixed and centrifuged briefly.

PROCEDURE:

1. The following reagents were added to a nuclease free 1.5ml centrifuge tube:
   a. 200µl of lysis buffer
   b. 5µl of carrier RNA
   c. 200µl of plasma
   d. 20µl of proteinase K
2. Centrifuge tube was mixed immediately by inverting it.
3. The tube was incubate at 56° C for 15min.
4. After taking the tube from incubator 300µl of 100% ethanol was added and mixed by a vortex for 30 seconds.

5. Then the tube was centrifuged for few seconds to bring down drops to bottom of the tube.
6. Entire sample was then transferred into the pure-fast spin column.
7. The pure fast spin column was centrifuge at 12000 rpm for 1 min.
8. The flow-through was discarded and the column was placed back into the same collection tube.
9. To this 500µl of 70% ethanol was added to the pure-fast spin column.
10. The pure fast spin column was centrifuge at 12000 rpm for 1 min.
11. The flow-through was discarded and the column was placed back into the same collection tube.
12. Empty spin column was attached with collection tube and centrifuged at 12000 rpm for an additional 2 min essentially to avoid residual ethanol.
13. After that the collection tube was discarded.
14. The pure fast spin column was transferred to a fresh 1.5ml centrifuge tube

15. To this, 50µl of elution buffer was added to the Centre of pure fast spin column membrane and incubate for 2 min at room temperature.
16. The pure fast spin column and the centrifuge tube was centrifuged at 12000rpm for 1 min and the pure fast spin column was then discarded.
17. Now the centrifuge tube containing the eluted nucleic acid was stored at -80°C for PCR analysis.

SEMI NESTED REVERSE TRANSCRIPTASE PCR REACTION

MATERIALS REQUIRED:

- 0.2ml PCR tubes
- Micro pipettes variable volume
- Sterile pipette tips with aerosol barrier
- Vortex mixer
- Centrifuge with rotor for 1.5ml reaction tubes

SAMPLE: extracted viral nucleic acid

All precautions to avoid PCR contamination were undertaken. Before use all the reagents were thawed completely, mixed and centrifuged briefly.

Semi nested Reverse transcriptase PCR was done in two steps

STEP1: c DNA synthesis

Master mix was prepared by the following reagents provided in the kit for one sample in a 1.5ml micro centrifuge tube.

- RT Mix = 6µl
- cDNA primer mix= 4 µl
- enzyme mix=0.5 µl

1. All of the above was spun down at 1000rpmfor 30 seconds.

2. 10.5 µl of the master mix was taken and 9.5 µl of the extracted viral nucleic acid was added to each PCR reaction tube.

3. Then it was incubated in a thermocyclerat 42°C for 45 minutes.

4. This step was critical to inactivate the reverse transcriptase

STEP2: PCR reaction set up

Master mix was prepared by the following reagents provided in the kit for one sample in a 1.5ml micro centrifuge tube.

- PCR master mix = 10µl
- Primer mix = 10 µl
- Water = 3 µl
- c DNA = 2 µl

PRIMER PREPARATION

The above reagents were mixed together and spun down at 1000rpm for 30 seconds to avoid loss of DNA pellets.

POSITVE CONTROL

23µl of the primer mix and 5µl of positive control in one PCR reaction tubes was taken.

NEGATIVE CONTROL

23µl of the primer mix and 2µl of nuclease free water in one PCR reaction tubes was taken.

SETTING UP OF PCR REACTION:

1. 23µl of the primer mix and 2 µl of the STEP-1 product in each PCR reaction tubes.
2. Positive control and negative control were also included.
3. The reaction vials were incubated in a thermocycler

- Initial denaturation: 95°C for 5 min
- 35 cycles of

  -Denaturation at 95°C – 30sec

  -Annealing at 60°C – 30sec

  -Extension 72°C - 30sec
- Final extension at 72°C – 5min

The primer sequence of the HEV targeted is

Forward:5’-GGAGCCATCACCTATGCCTTATGT-3'

Reverse Primer: 5'-ACGGGAGCAGCAAAAGGCTTG-3'

ANALYSIS BY GEL ELECTROPHORESIS

PREPARATION OF AGAROSE GEL

2% agarose gel was prepared by adding two grams of agarose powder to 100 ml of electrophoresis buffer, then heated in a microwave oven for 2 min, mixed until the agarose was evenly dissolved. After cooling to about 60 °C 5 µl of ethidium bromide was added to 100 ml of the gel to enable visualization of DNA after electrophoresis. Ethidium bromide being carcinogen being handled with precaution.

A well-formed COMB/template was placed across the end of the casting tray which was covered with a cellophane tape in its edges and the freshly prepared gel was poured into the casting tray which act as a mold. This was let to solidify at room temperature.

PREPARATION OF ELECTROPHORETIC BUFFER

To one litre of distilled water 20ml of Electrophoresis (TAE-Tris Acetic acid –EDTA) buffer was added and freshly prepared. **GEL**

GEL ELECTROPHORESIS

Electrophoresisbuffer was poured into the electrophoresis chamber. After the gel has hardened enough, the gel was gently placed on the electrophoresis tank and was made sure that the gel was completely immersed in the solution and comb was removed carefully from the gel.

10µl of DNA ladder, sample, negative control and positive controls and were loaded into the gel wells using micropipette.

Electrical leads were connected to the electrophoresis tank. And the current was supplied with a voltage 50-100V so that negatively charged DNA migrate from cathode to anode. Gel running time was approximately the time taken by the gel loading dye to cover three fourth of distance in the gel.

After the gel electrophoresis, PCR products were observed using UV transilluminator.

INFERENCE:

Ladder and positive control were formed appropriately.

No amplicons of size 200bp was seen.

Hence, no viral RNA was detected

OBSERVATION AND RESULTS

Two hundred asymptomatic pregnant women attending routine antenatal clinic in a tertiary care hospital, were included in the Prospective study. Blood samples were collected and serum were separated by centrifuging at 2500 rpm. All the serum samples were stored in two sets to detect IgM, IgG antibodies against hepatitis E virus and to detect HEV RNA by seminested PCR in the department of Microbiology from January 2014 to September 2014. The results were analysed as follows.

TABLE-1: AGE DISTRIBUTION

Trimester	Age (n= 200)				
	16-20	21-25	26-30	31-35	TOTAL
1st Trimester	4	14	7	1	26
2nd Trimester	15	34	31	7	87
3rd Trimester	12	44	24	7	87
Total	31	92	62	15	200
%	15.5%	46.0%	31.0%	7.5%	100%
P Value	0.733				
Mean Age	24.55				
Std. Deviation	3.838				

Commonest age group was between 21-25 years of age.

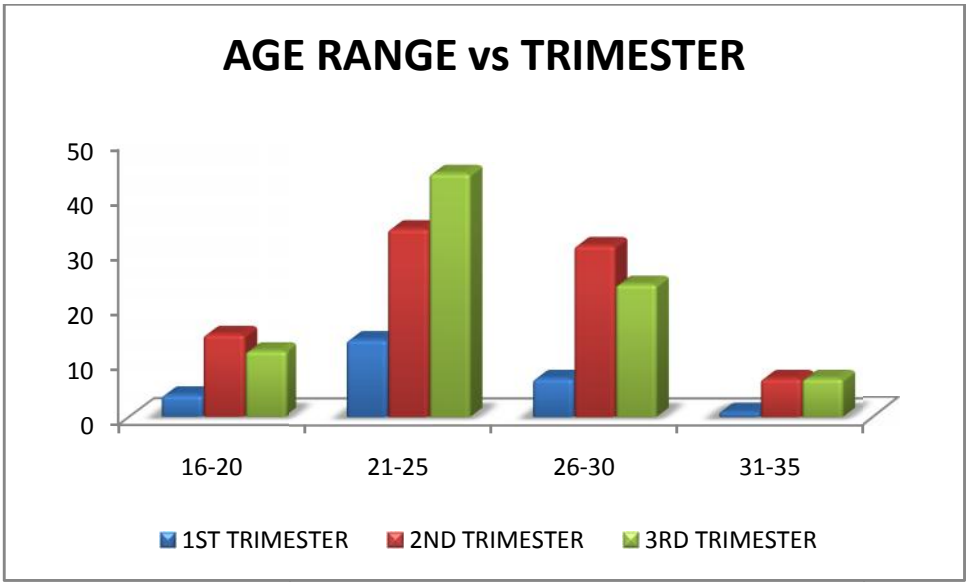

AGE RANGE vs TRIMESTER
50
40
30
20
10
0
16-20
21-25
26-30
31-35
1ST TRIMESTER
2ND TRIMESTER
3RD TRIMESTER

TABLE 2: GRAVIDA

Trimester	Gravida			
	Primi	2nd Gravida	3rd Or Multi Gravida	Total
1st Trimester	11	9	6	26
2nd Trimester	44	31	12	87
3rd Trimester	43	31	13	87
Total	98	71	31	200
%	49%	35.5%	15.5%	100%
P value	0.838			
Df	4			

Majority of the pregnant women were Primigravida (49%).

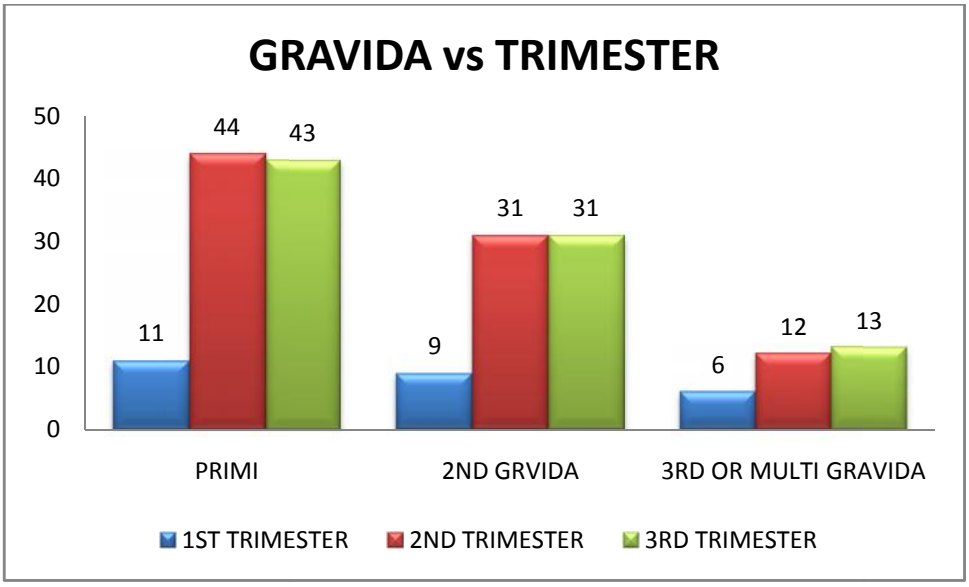

GRAVIDA vs TRIMESTER
50
40
30
20
10
0
44
43
31
31
11
9
12
13
6
PRIMI
2ND GRVIDA
3RD OR MULTI GRAVIDA
1ST TRIMESTER
2ND TRIMESTER
3RD TRIMESTER

TABLE: 3. EDUCATIONAL STATUS

	Educational Status						
Trimester	Illiterate	Primary School	Middle School	High School	Diploma	Graduate	Total
1st Trimester	3	1	12	4	2	4	26
2nd Trimester	11	13	33	20	1	9	87
3rd Trimester	12	8	40	13	0	14	87
Total	26	22	85	37	3	27	200
%	13.0%	11.0%	42.5%	18.5%	1.5%	13.5%	100%
P Value	O.156						
Df	10						

Most of the pregnant women have studied up to middle school (42.5%) followed by high school (18.5%)

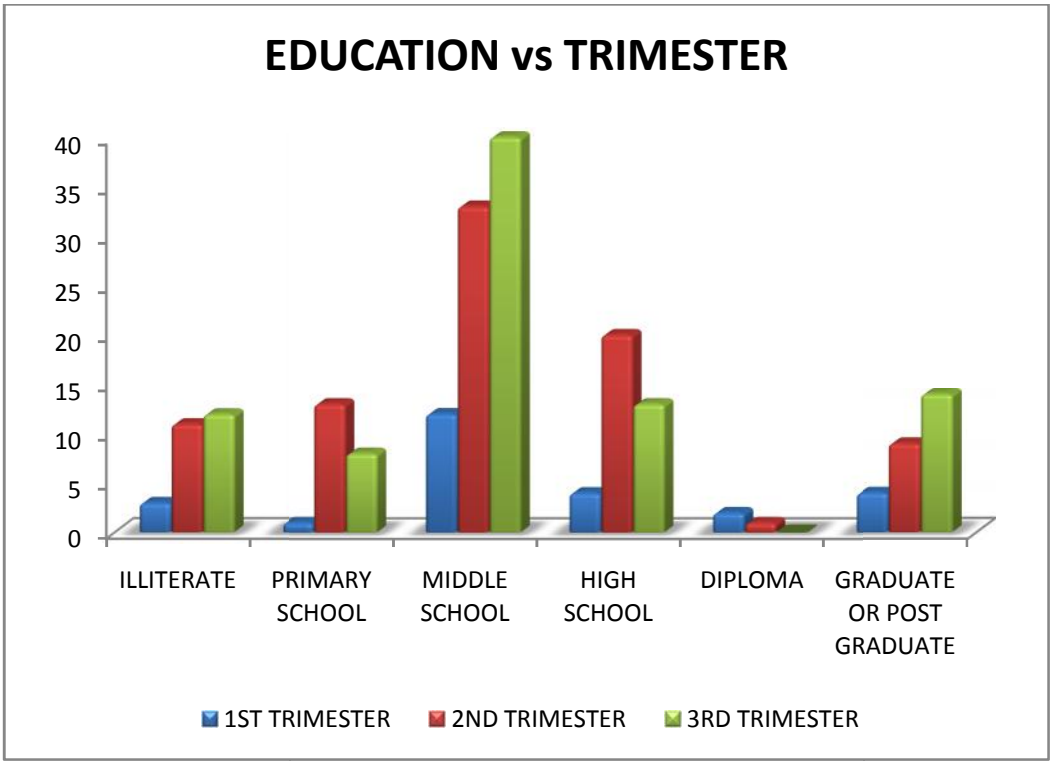
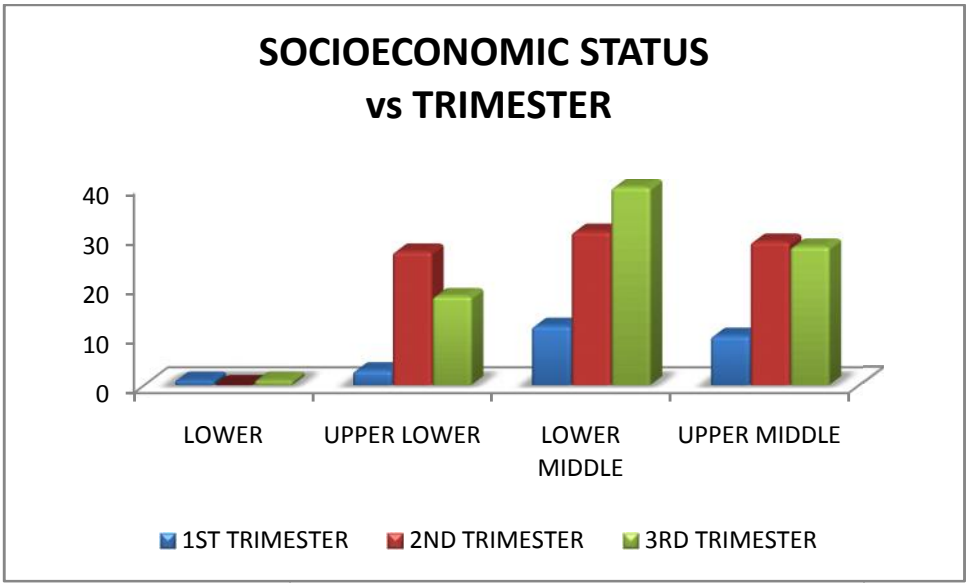

EDUCATION vs TRIMESTER
40
35
30
25
20
15
10
5
0
ILLITERATE
PRIMARY SCHOOL
MIDDLE SCHOOL
HIGH SCHOOL
DIPLOMA
GRADUATE OR POST GRADUATE
1ST TRIMESTER
2ND TRIMESTER
3RD TRIMESTER

TABLE: 4. SOCIOECONOMIC STATUS AND OCCUPATION

Trimester	Socio Economic Status					Occupation		
	Lower	Upper Lower	Lower Middle	Upper Middle	Total	Working Outdoor	Indoor House work	Total
1st Trimester	1	3	12	10	26	6	20	26
2nd Trimester	-	27	31	29	87	7	80	87
3rd Trimester	1	18	40	28	87	5	82	87
Total	2	48	83	67	200	18	182	200
%	1.0%	24.0%	41.5%	33.5%	100%	9.0%	91.0%	100%
P Value	0.211					***0.023***		
Df	6					2		

Majority of the pregnant women was in the lower middle class (41.5%) and 91.0% of them were not working


SOCIOECONOMIC STATUS
vs TRIMESTER
40
30
20
10
0
LOWER
UPPER LOWER
LOWER MIDDLE
UPPER MIDDLE
1ST TRIMESTER
2ND TRIMESTER
3RD TRIMESTER

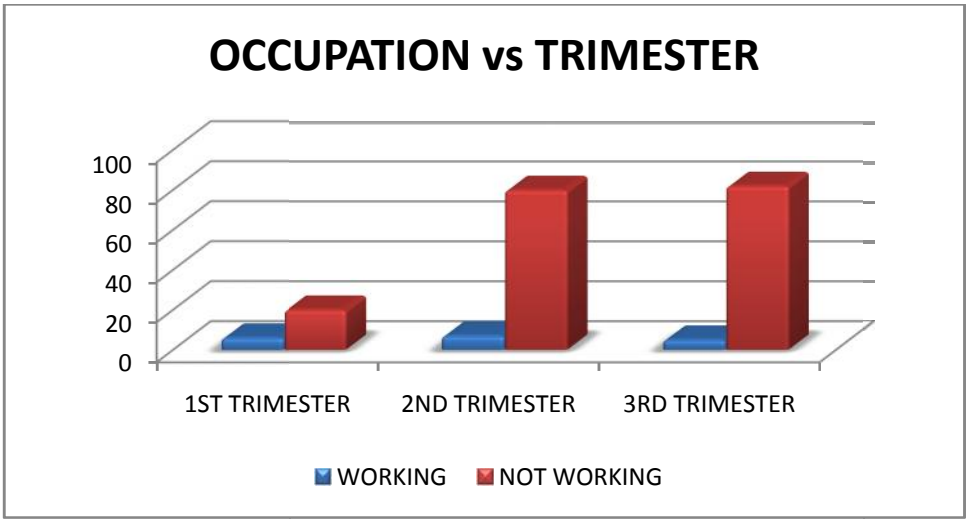

OCCUPATION vs TRIMESTER
100
80
60
40
20
0
1ST TRIMESTER
2ND TRIMESTER
3RD TRIMESTER
WORKING
NOT WORKING

TABLE: 5.OVERCROWDING AND FOOD HABIT

Trimester	Over Crowding		Food Habit	
	Yes	No	Veg	Non-Veg
1st Trimester	6	20	4	22
2nd Trimester	29	58	5	82
3rd Trimester	26	61	5	82
Total	61	139	14	186
%	30.5%	69.5%	7.0%	93.0%
P Value	0.6		0.199	
Df	2		2	

30.5% of the pregnant women resides in an overcrowded house and 93.0% of them were following non-veg diet.

TABLE: 6. DRINKING WATER AND USE OF BOILED WATER

	1st Trimester	2nd Trimester	3rd Trimester	Total	%
Source Of Drinking Water					
Aqua	1	0	1	2	1.0
Can	2	5	8	15	7.5
Lorry	1	17	17	35	17.5
Muni	14	48	37	99	49.5
Pump	6	12	13	31	15.5
Sump	2	2	4	8	4.0
Tap Out	0	2	7	9	4.5
Well	0	1	0	1	0.5
Total	26	87	87	200	100%
P Value	0. 258				
Df	14				
Use of boiled water					
Yes	6	22	21	49	24.5
No	20	65	66	151	75.5
P value	0.064				
Df	2				

Majority (49.5%) of the pregnant women used municipal water available at their home and 75.5% of them did not used boiled water

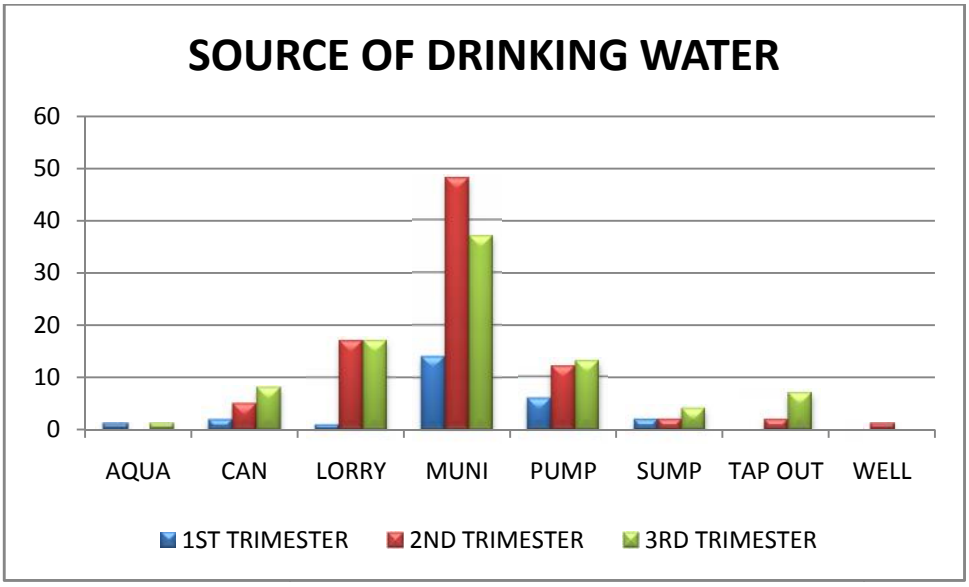

SOURCE OF DRINKING WATER
60
50
40
30
20
10
0
AQUA
CAN
LORRY
MUNI
PUMP
SUMP
TAP OUT
WELL
1ST TRIMESTER
2ND TRIMESTER
3RD TRIMESTER

TABLE: 7. TOILET FACILITY AND USE OF SOAP FOR HAND WASHING

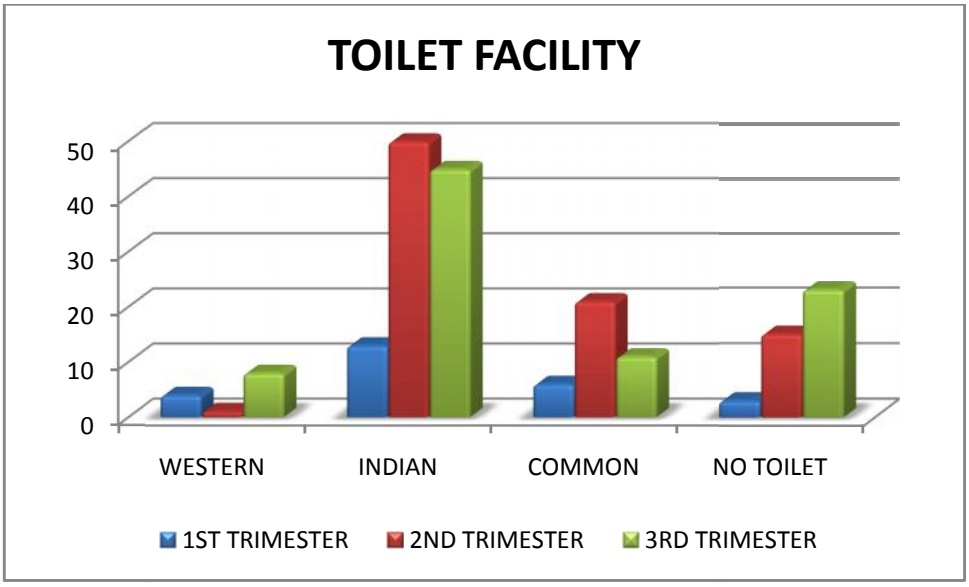
Trimester	Toilet Facility					Use Of Soap	
	Western	Indian	Common	No Toilet	Total	Yes	No
1st Trimester	4	13	6	3	26	16	10
2nd Trimester	1	50	21	15	87	56	31
3rd Trimester	8	45	11	23	87	62	25
Total	13	108	38	41	200	134	66
%	6.5%	54.0%	19.0%	20.5%	100%	67.0%	33.0%
P Value	0.024					0.512	
Df	6					2	

Common toilet- Indian type

54.0% of pregnant women commonly used Indian toilet

20.5% of them had no toilet facility and used open field for defecation.

Majority of them were using soap for hand washing ie., 67%


TOILET FACILITY
50
40
30
20
10
0
WESTERN
INDIAN
COMMON
NO TOILET
1ST TRIMESTER
2ND TRIMESTER
3RD TRIMESTER


USE OF SOAP FOR HAND WASHING AFTER USING TOILET
70
60
50
40
30
20
10
0
YES
NO
1ST TRIMESTER
2ND TRIMESTER
3RD TRIMESTER

TABLE: 8. PROBABLE RISK FACTORS

Clinical Data	1st Trimester N=26		2nd Trimester N=87		3rd Trimester N=87		Total N=200	
H/O Blood Transfusion	-	-	10	11.5%	6	6.9%	16	8%
H/O jaundice In The Patient	4	15.4%	15	17.2%	11	12.6%	30	15%
H/O Family Of jaundice	2	7.7%	3	3.4%	2	2.3%	7	3.5%

H/O blood transfusion was more commonly seen in the 2nd trimester of pregnancy

Both H/O jaundice and family H/O jaundice was present in pregnant women in all three trimester

Above probable risk factors were statistically not significant.

TABLE: 9. BLOOD GROUPS

Blood Group	Trimester			Total	%
	1ST	2ND	3RD		
A-	0	1	1	2	1.0%
A+	7	8	20	35	17.5%
AB+	2	6	6	14	7.0%
B-	0	4	1	5	2.5%
B+	5	20	20	45	22.5%
O-	1	3	3	7	3.5%
O+	11	45	36	92	46.0%
Total	26	87	87	200	100%
P value	0.561				
Df	12				

Majority of the pregnant women had O+ blood group (46.0%) followed by 22.5% of B+ women. Other blood groups were in the range of 1% to 17 %.

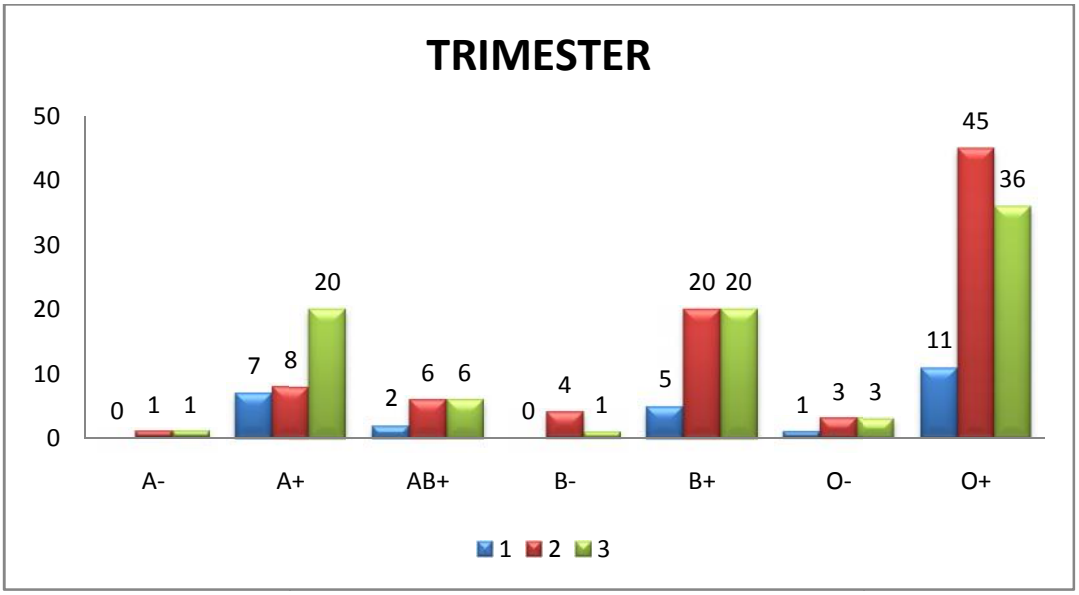

TRIMESTER
50
40
30
20
10
0
0 1 1
7 8 20
2 6 6
0 4 1
5 20 20
1 3 3
11 45 36
A-
A+
AB+
B-
B+
O-
O+
1 2 3

TABLE: 10. TIMESTER AND RESULTS OF ELISA AND REVERSE TRANSCRIPTASE PCR

Trimester	ELISA			RT-PCR
	Only IgM + n =5	Only IgG + n = 11	Both IgM+ & IgG+ n = 7	HEV-RNA
1st Trimester	0	1	3	-
2nd Trimester	3	5	2	-
3rd Trimester	2	5	2	-
Total	5	11	7	-
%	2.5%	5.5%	3.5%	-

Over all IgM+ (5 + 7 = 12) ------- 6%

Over all IgG+ (11 + 7 = 18) ------- 9%

No cases were found to be positive for HEV-RNA by Reverse transcriptase-PCR.

TABLE: 11 AGE AND HEV POSITIVITY

Age	Only IgM+	%	Only IgG+	%	Both IgM+IgG+	RT-PCR HEV-RNA
16- 20	1	20.0%	-	-	-	-
21-25	-	-	3	27.3%	4	-
26-30	4	80.0%	7	63.6%	3	-
31-35	-	-	1	9.1%	-	-
Total	5	100%	11	100%	7	-
P value	is 0.079		0.081		0.533	
df	3		3		3	

Majority of the positive results of ELISA were seen in the age group 26-30.

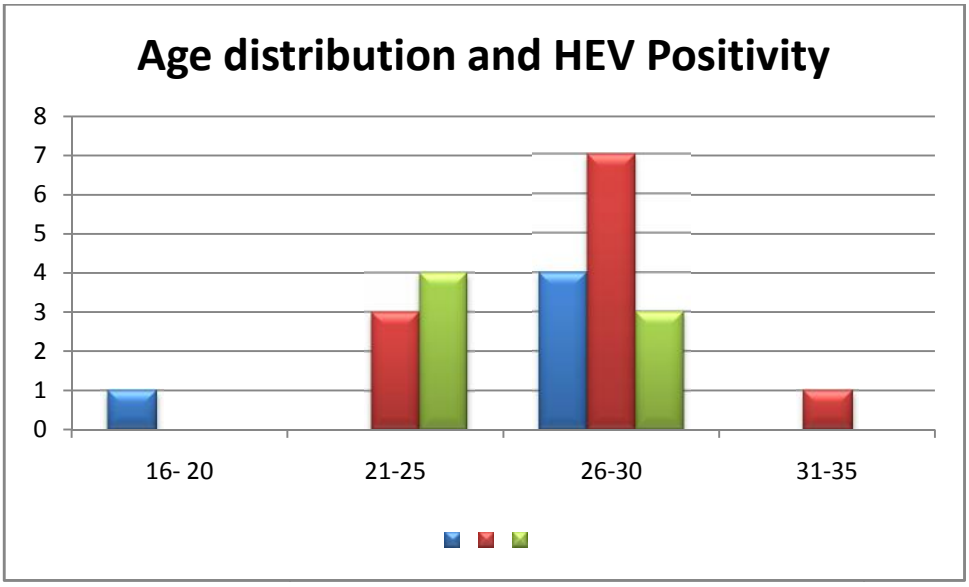

Age distribution and HEV Positivity
8
7
6
5
4
3
2
1
0
16- 20
21-25
26-30
31-35

TABLE: 12. GRAVIDA AND HEV POSITIVITY

Number Of Pregnancy	ELISA						RT-PCR
	IgM+		IgG+		Both IgM+& IgG+		HEV-RNA
Primi	1	20%	3	27.3%	3	42.9%	-
2nd Gravida	3	60%	6	54.5%	3	42.9%	-
3rd Or Multi Gravida	1	20%	2	18.2%	1	14.3%	-
Total	5	100%	11	100%	7	100%	-

Seropositivity were more commonly seen the 2nd gravida.

HEV positivity cases were equal equally distributed between primigravida and multigravida.

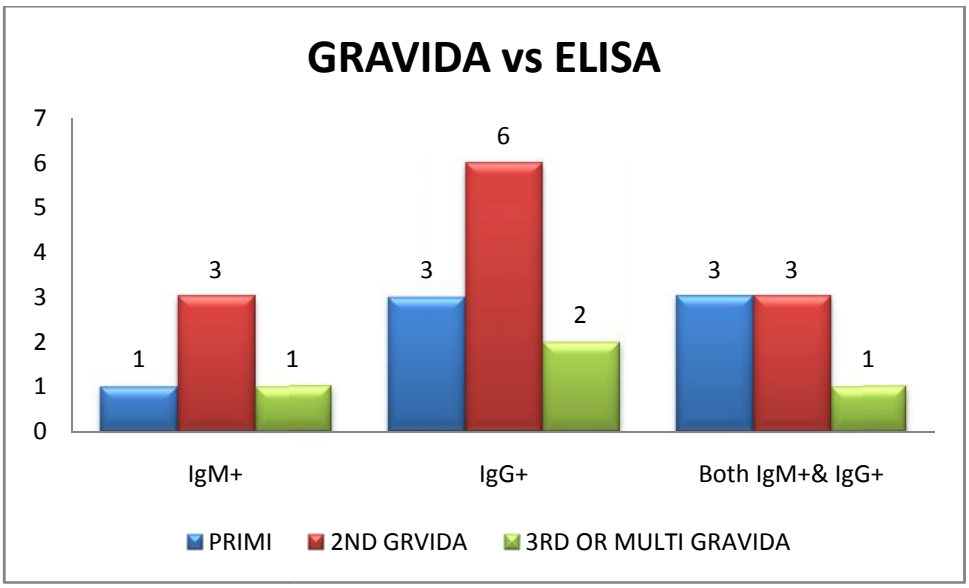

GRAVIDA vs ELISA
7
6
5
4
3
2
1
0
1
3
1
3
6
2
3
3
1
IgM+
IgG+
Both IgM+& IgG+
PRIMI
2ND GRVIDA
3RD OR MULTI GRAVIDA

TABLE: 13. EDUCATIONAL STATUS AND HEV POSITIVITY

Educational Status	ELISA						RT-PCR
	Only IgM+	%	Only IgG+	%	Both IgM+ IgG+	%	HEV RNA
Illiterate	-	-	1	9.1%	-	-	-
Primary School	1	20%	3	27.3%	3	42.0%	-
Middle School	3	60%	3	27.3%	4	57.1%	-
High School	1	20%	-	-	-	-	-
Diploma	-	-	-	-	-	-	-
Graduate	-	-	4	36.4%	-	-	-
Total	5	100%	11	100%	7	100%	-
P Value	0.822		0.064		0.064		-
Df	5		5		5		-

Majority of the IgM and IgG positive cases were in the middle school (57.1%) followed by primary school (42.0%).

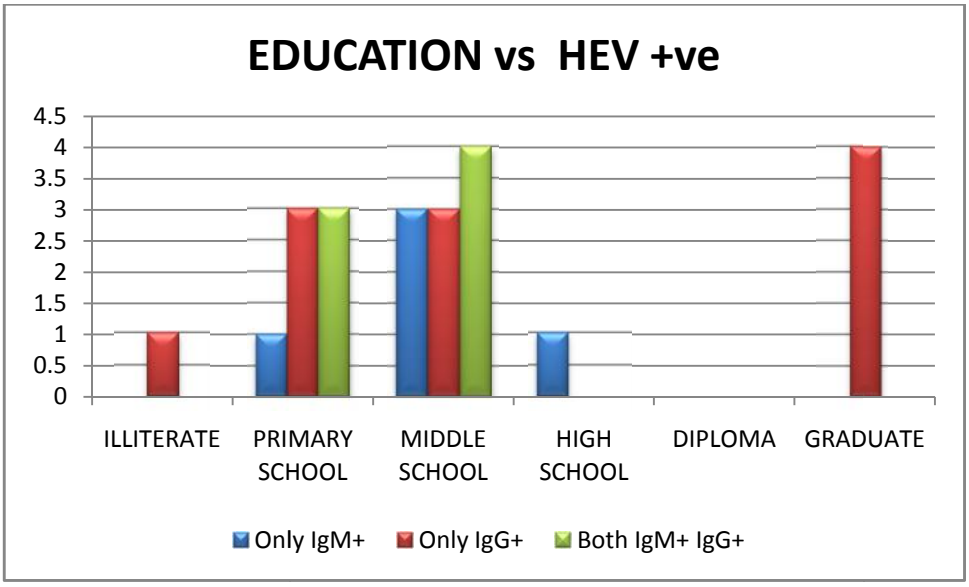

EDUCATION vs HEV +ve
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
ILLITERATE
PRIMARY SCHOOL
MIDDLE SCHOOL
HIGH SCHOOL
DIPLOMA
GRADUATE
Only IgM+
Only IgG+
Both IgM+ IgG+

TABLE: 14. SOCIOECONOMIC STATUS, OCCUPATION AND HEV POSITIVITY

Socioeconomic Status	ELISA						PCR
	Only IgM+		Only IgG+		Both IgM+IgG+		HEV-RNA
Socio Economic Status							
Lower	-		-		-	-	-
Upper Lower	2	40%	5	45.5%	4	57.1%	-
Lower Middle	2	40%	3	27.3%	3	42.9%	-
Upper Middle	1	20%	3	27.3%	-	-	-
Total	5	100%	11	100%	7	100%	-
P Value	0.880		3.051		5.826		
Df	3		3		3		
Occupation							
Working	-	-	2	18.2%	-		-
Indoor Housework	5	100%	9	81.8%	7	100%	-
Total	100%	100%	11	100%	7	100%	-
P Value	0.507		1.198		0.717		
Df	1		1		1		

Most of the HEV positivity were seen in the upper lower (57.1%) and lower middle class (42.9%). All seropositive cases were seen in the indoor housework group

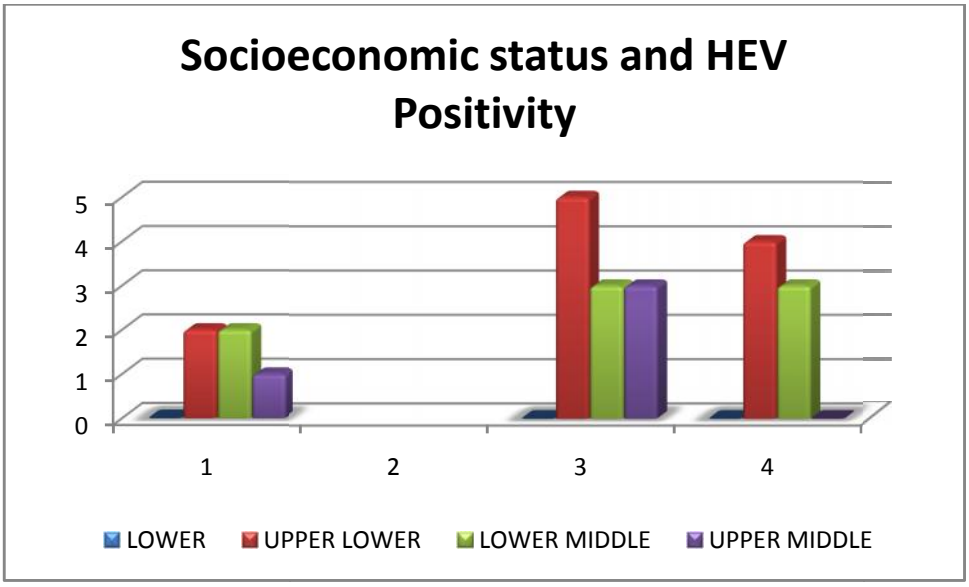

Socioeconomic status and HEV Positivity
5
4
3
2
1
0
1
2
3
4
LOWER
UPPER LOWER
LOWER MIDDLE
UPPER MIDDLE

TABLE: 15. OVERCROWDING AND FOOD HABIT WITH HEV POSITIVITY

Over Crowding	ELISA						PCR HEV RNA
	Only IgM+		Only IgG+		Both IgM+ IgG+		
Yes	3	60%	3	27.3%	5	51.4%	-
No	2	40%	8	72.7%	2	28.4%	-
Total	5	100%	11	100%	7	100%	-
P Value	0.147		**0.002**		**0.017**		
Df	1		1		1		
Food Habit							
Veg	-	-	1	9.1%	1	14.3%	-
Non-Veg	5	100%	10	90.9%	6	85.7%	-
Total	5	100%	11	100%	7	100%	-
P Value	0.534		0.349		0.460		
Df	1		1		1		

Overcrowding and pregnant women having non-vegetarian diet were seen to have most of the positive cases.

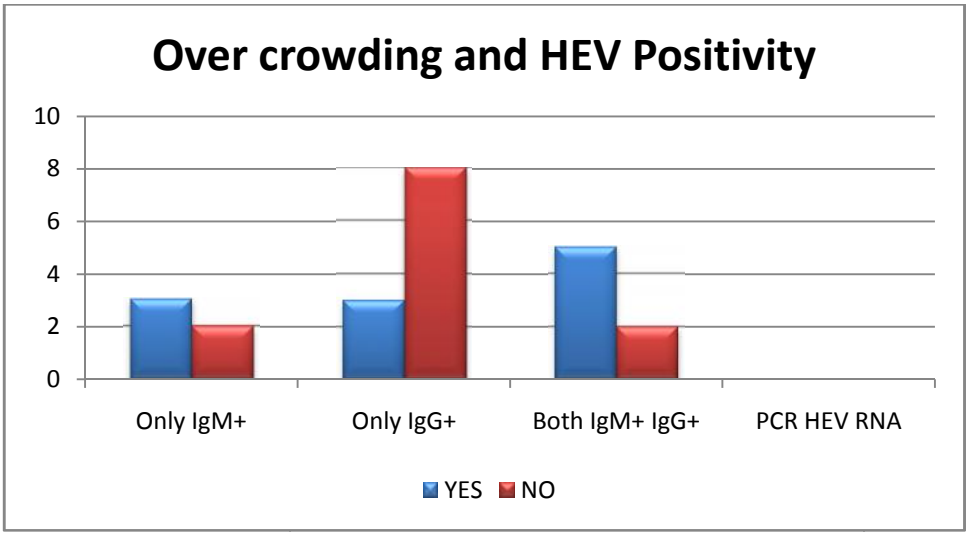
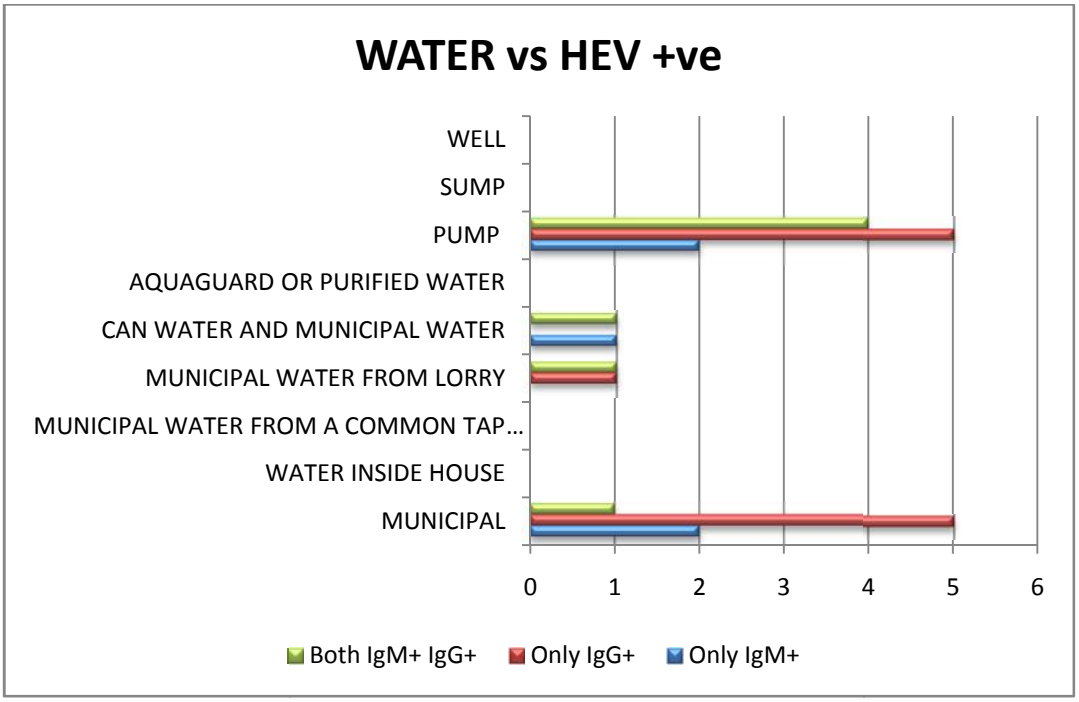

Over crowding and HEV Positivity
10
8
6
4
2
0
Only IgM+
Only IgG+
Both IgM+ IgG+
PCR HEV RNA
YES
NO

TABLE: 16. SOURCE OF DRINKING WATER AND USE OF BOILED WATER WITH HEV POSITIVITY

Source of water	ELISA						RT-PCR
	Only IgM+		Only IgG+		Both IgM+ IgG+		HEVRNA
Drinking water facility							
Municipal waterInside House	2	40%	5	45.5%	1	14.3%	-
Municipal Water CommonTap	-	-	-		-		-
Municipal Water From Lorry	-	-	1	9.1%	1	14.3%	-
Municipal Water Can Water And	1	20%	-		1	14.3%	-
Aqua guard Or Purified Water	-		-		-		-
Pump	2	40%	5	45.5%	4	57.1%	-
Sump			-				-
Well							-
Total	5	100%	-	100%	7	100%	-
P Value	0.714		0.233		0.132		
Df	7		7		7		
Use Of Boiled Water							
Yes	2	40%	2	18.2%	1	14.3%	-
No	3	60%	9	81.8%	6	85.7%	-
Total	5	100%	11	100%	7	100%	-
P Value	0.414		0.616		0.522		
Df	1		1		1		

Most of the positive cases were found in the people drinking pump water and majority were not using boiled water.


WATER vs HEV +ve
WELL
SUMP
PUMP
AQUAGUARD OR PURIFIED WATER
CAN WATER AND MUNICIPAL WATER
MUNICIPAL WATER FROM LORRY
MUNICIPAL WATER FROM A COMMON TAP...
WATER INSIDE HOUSE
MUNICIPAL
0
1
2
3
4
5
6
Both IgM+ IgG+
Only IgG+
Only IgM+

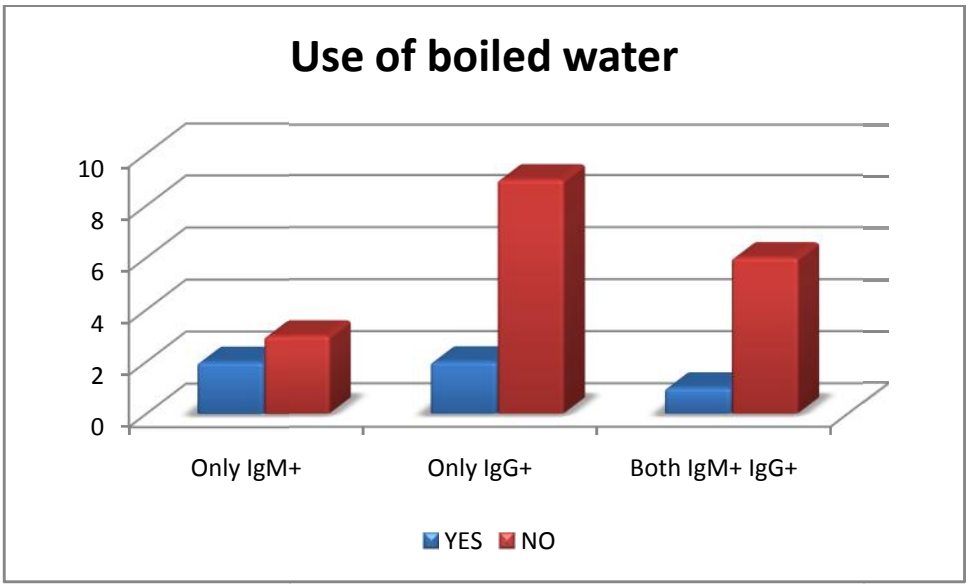
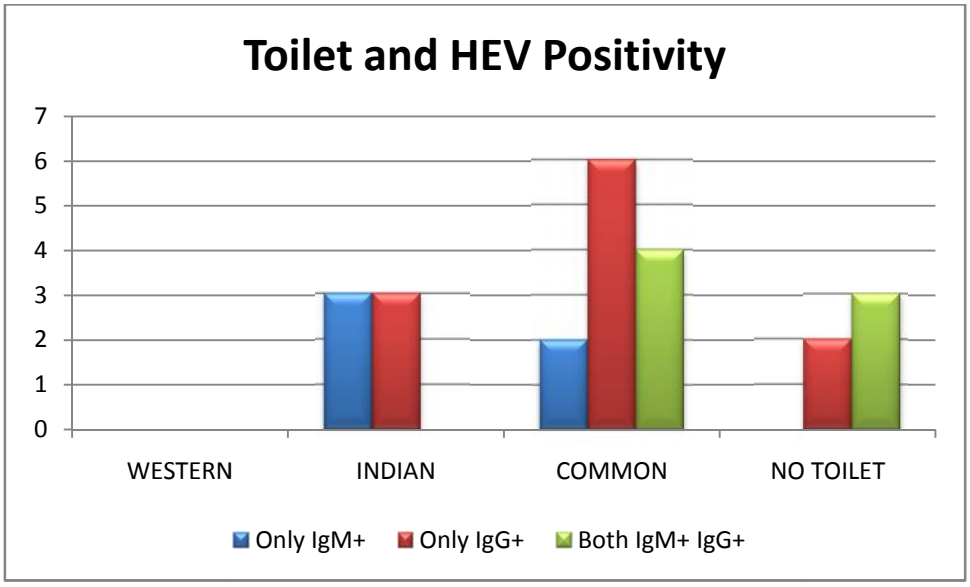

Use of boiled water
10
8
6
4
2
0
Only IgM+
Only IgG+
Both IgM+ IgG+
YES
NO

TABLE: 17.TOILET FACILITY AND USE OF SOAP WITH HEV POSITIVITY

Toilet Facility	ELISA						PCR HEV-RNA
	IgM+		IgG+		Both IgM+ & IgG+		
Western	-	-	0	-	0	0	-
Indian	3	60%	3	27.3%	0	0	-
Common	2	40%	6	54.5%	4	57.1%	-
No Toilet	0	-	2	18.2%	3	42.9%	-
Total	5	100%	11	100%	7	100%	-
P Value	0.456		0.018		0.008		-
Df	3		3		3		-
Use Of Soap For Hand Washing							
Yes	1	20%	2	18.2%	0	0	-
No	4	80%	9	81.8%	7	100%	-
P Value	0.024		0.000		0.000		-
Df	1		1		1		-

HEV Positivity were more commonly seen in pregnant women using Indian type common toilets (57.1%) and without toilet facility (47.9%)

Use of soap and HEV positivity were significantly associated


Toilet and HEV Positivity
7
6
5
4
3
2
1
0
WESTERN
INDIAN
COMMON
NO TOILET
Only IgM+
Only IgG+
Both IgM+ IgG+

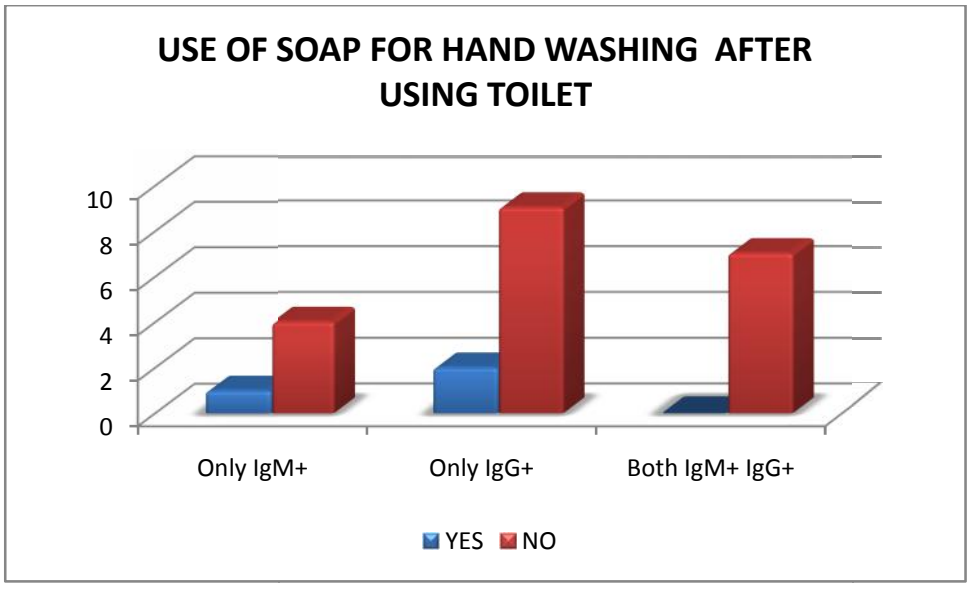

USE OF SOAP FOR HAND WASHING AFTER USING TOILET
10
8
6
4
2
0
Only IgM+
Only IgG+
Both IgM+ IgG+
YES
NO

TABLE:18. PROBABALE RISK FACTORS AND HEV POSITIVITY

Clinical Data	Only IgM+	Only IgG+	Both IgM+ IgG+	HEV-RNA
H/O Blood Transfusion	2	-	-	-
H/O Hepatitis In The Patient	1	-	2	-
H/O Family Hepatitis	-	-	-	-

No significance was found between H/O blood transfusion, H/O jaundice and family H/O jaundice and HEV positivity.

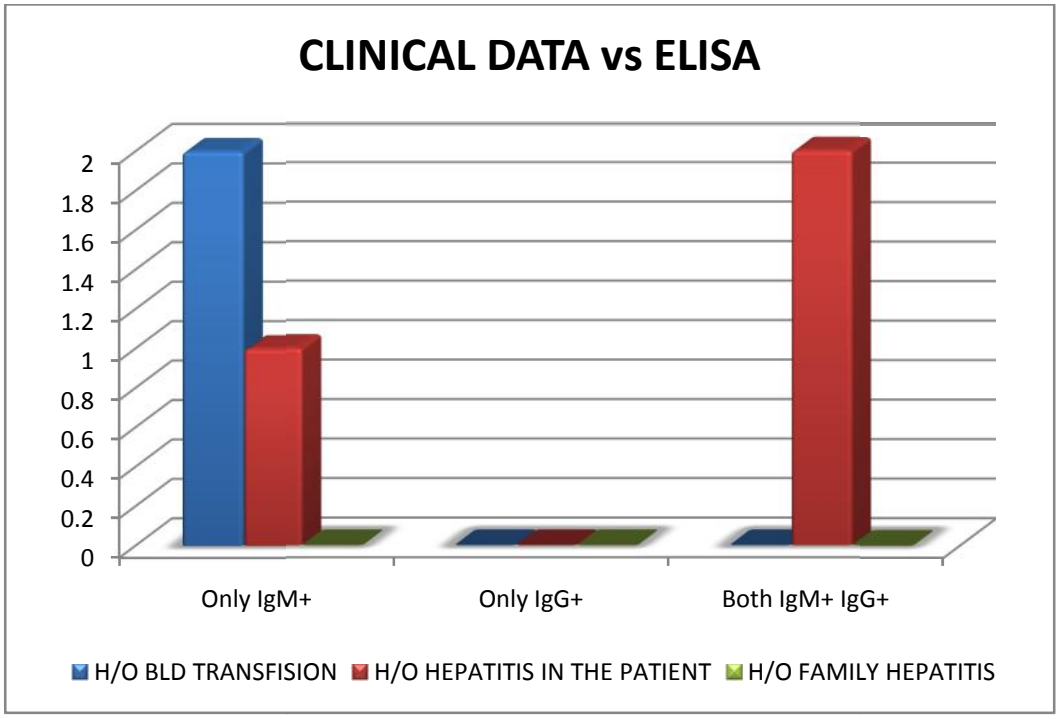

CLINICAL DATA vs ELISA
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Only IgM+
Only IgG+
Both IgM+ IgG+
H/O BLD TRANSFISION
H/O HEPATITIS IN THE PATIENT
H/O FAMILY HEPATITIS

TABLE: 19. BLOOD GROUPS AND HEV POSITIVITY

Blood Group	ELISA			Total	PCR HEV RNA
	Only IgM+	Only IgG+	Both IgM+ IgG+		
A+	2	2	-	4	-
B+	-	2	-	2	-
B-	-	-	1	1	-
AB+	-	2	1	3	-
O+	2	5	4	11	-
0-	-	-	-		-

HEV positivity and blood group were not associated

DISCUSSION

Earlier to the development of appropriate laboratory assays, clinical hepatitis E virus infection was considered by the exclusion of hepatitis A virus and hepatitis B virus (hence the designated as NANB). Original methods for the detection of HEV infection involved immune electron microscopy of stool or bile samples and fluorescent antibody blocking technique to detect anti-HEV from serum samples and liver sections of the patient. Both of these methods had very limited sensitivity for the detection of hepatitis E virus both in acute and remote infections and also expensive and laborious hence not useful for routine diagnosis.

Enzyme Linked Immunosorbent Assay (ELISA) is the serological test available for antibody detection of both anti HEV IgM and IgG of hepatitis E virus infection. It is highly sensitive, costeffective and a practical method for detection of HEV antibodies

Reverse Transcriptase PCR is a nucleic acid amplification based technique. It is the method of choice for the specific detection of HEV RNA in blood and faeces. The test is of importance during the window period as the specific antibodies to HEV are not mounted to the detectable levels, hence the serological test are found to be negative[3].

The population included in this study were asymptomatic pregnant women who comes for routine antenatal check-up in a tertiary care hospital. All patients were from different areas in and around Chennai.

Sera of all the enrolled patients were analysed for the presence of anti-HEV IgM and IgG antibody against hepatitis E virus by a commercially available Enzyme Linked Immunosorbent Assay (**DSI-EIA-ANTI-HEV-M; DSI-EIA-ANTI-HEV-G**) and HEV RNA was detected by semi nested Reverse transcriptase PCR (**HELINI BIOMOLECULES**).

AGE DISTRIBUTION (TABLE:1)

The study group included in asymptomatic pregnant mothers were aged 16-35 years with the mean age of 24.55 years. According to the study carried out by Shams et al, out of 65 pregnant women, the mean age was 25 years[60] and HEV seroprevalence seemed to be high among 21-25 years of age (Andrew A Agjei et al)[23]. In the present study, majority of the cases were in the age group of 21 to 25 (46%) followed by 62(31%) cases in the age group 26 to 30 years.

GRAVIDA (TABLE: 2)

Among 200 asymptomatic pregnant patients, 98 (49%) were primigravida, 71 (35.5%) of them were 2nd gravida and 31 (15.5%) were 3rd or multi gravida. Compared to primigravida, 2nd and multi gravida were less in number. Most of them were in their second and third trimester of pregnancy. According to Shams et al, HEV affects more commonly the Primigravida and in the last trimester of pregnancy[60]. According to MojtabaRasti et al, there was a significant diference between seropositvity and different trimester of pregnancy and most of the positive were occurred in the third trimester of pregnancy[61]. According to Nargis Begum et al mean period of gestation was 19.06 ±2.25 wk with a range of 16-24 wk[2].

SOCIOECONOMIC STATUS AND OCCUPATION (TABLE:4)

In this study according to the Kuppuswamy's criteria, following were the distribution of socioeconomic status:

Lower class - 2 (1.0%)

Upper Lower class - 48 (24.0%)

Lower Middle class - 83 (41.5%)

Upper Middle class - 67 (33.5%)

Majority of them were in the lower middle class. According to Nargis begum et al lower socioeconomic status is the only main risk factor associated with HEV IgG antibody[2].

About 182 (91.0%) of them were indulged in indoor house work and only 18 (9.0%) were outdoor working. This results were consistent with the study done by Alain B. Labrique et al [56]in which > 89% of the women reported their occupation as indoor housework.

OVER CROWDING AND FOOD HABIT (TABLE: 5)

According to Bronfman'c criteria for assessing overcrowding [55](i.e., more than 3.5 person per living room), about 61 (30.5%) of the pregnant women resides in an overcrowded house. Majority of them were in the 2nd and 3rd trimester of pregnancy. According to Cosme et al hepatits E sero prevalence was significantly associated with overcrowding[55].

Asymptomatic pregnant women 186 (93%) of the pregnant women were following non-vegetarian diet whereas only 14 (7.0%) of them were vegetarian. Rakesh et al[4] has shown similar results as that of our present study.

DRINKING WATER AND USE OF BOILED WATER (TABLE: 6)

99 (49.5%) of the pregnant were using municipal water which was available inside their home when compared to 9 (4.5%) of pregnant women who get water from a common tap in the street. 35 (17.5%) of them get municipal water through lorry transportation. 15 (7.5%) and 2 (1%) of them use can water and purified water (using aqua guard purifier) in addition to municipal water respectively. According to Zimaity et al, patients residing in homes that were not connected to a municipal source for drinking water appeared to have increased risk for HEV infection [58].

49 (24.5%) of the pregnant women were using boiled water whereas majority ie., 151 (75.5%) of them used water without boiling. Cosmeet al[55] studied that there was an association with use of boiled water and HEV infectivity.

TOILET FACILITY AND USE OF SOAP (TABLE: 7)

108 (54.0%) of the pregnant women commonly used Indian toilet. 38 (19.0%) and 13 (6.5%) of them were using common Indian

toilet and western toilet respectively.41 (20.5%) of them had no toilet facility and were using open field for defecation.

According to Surajudeen et al type of toilet facility was significantly associated with HEV seroprevalence and found to be more common among people who used open field for deafecation[59].

Majority of them were using soap for hand washing i.e., 134 (67%) and remaining 66 (33%) did not have the habit of use of soap for hand washing. Statistically significant value was found between hand washing and HEV positivity in a study by Surajudeenet al[59].

CLINICAL DATA (TABLE: 8)

16 (8%) of the pregnant women had history of blood transfusion and all of them were in 2nd and 3rd trimester of pregnancy. **30 (15%)** and **7 (3.5%)** of them had history of jaundice in the patient and family history of jaundice respectively.

BLOOD GROUP (TABLE: 9)

Majority of the pregnant women had O+ blood group followed by B+ blood group individuals. Other blood groups were between the range of 1% to 7%

SEROPREVALENCE OF HEV POSITIVITY (TABLE: 10)

In the present study, IgM and IgG seropositivity of asymptomatic pregnant women were **12 (6%)** and **18 (9%)** respectively. Among them only IgM+ cases were **5 (2.5%),** only IgG+ cases were **11 (5.5%)** and both IgM+&IgG+ were **7 (3.5%)**.

The results of the present study were similar to 5.62% seroprevalence of IgG, a report given by Daniel et al (2004) at Vellore from 600 samples including blood donors, antenatal mothers and from pre-operative patience[29,2].

But this results were much lower than 33.67% seroprevalence of IgG antibodies in a hospital based study, from north India[2]. And other studies from Pune and Lucknow documented a much higher prevalence in adult population (40%-50%). But the seroprevalence of this study is also comparable to the 3.6% seroprevalence of hepatitis E in pregnant women in Madrid, Spain and 6.6% seroprevalence of IgG antibodies to HEV reported in pregnant women infected with HIV in Gabon and central Africa[55].

AGE AND HEV POSITIVITY (TABLE: 11)

HEV positivity of pregnant women was seen most commonly in 26-30 fears when compared 21-25 years. Seropositivity of

asymptomatic pregnant women, appeared to increase as the age increases. The above observation was similar to the study results of Cosme et al, in which he states that there is an increase in seroprevalence of HEV with age in the pregnant women[55] .

GRAVIDA AND HEV POSITIVITY (TABLE:12)

When the seropositivity of pregnant women of different gravida were compared, majority was observed during their second gravida. In Primigravida, the seropositivity was 7 (30.4%) which was much less than the seropositivity of 2nd gravida. This result was similar to the study done by Cosme et al, where seropositivity of hepatitis E were in association with number of pregnancythough the reason was not yet clear[55].

EDUCATIONAL STATUS AND HEV POSITIVITY(TABLE: 13 and 3)

Most of the asymptomatic pregnant women (42.5%) in the study group have studied up to middle school. Majority of the IgM+ cases and IgG+ cases were seen in the middle school followed by the primary school. But educational status and HEV positivity were not statistically significant.

This present study results were similar according to Cosme et al observation, in which he states that there was no positive association

with the educational status and HEV seropositivity[55]. But Sekan et al study showed education seemed to be the only risk factor for HEV seroprevalence[18,58].

SOCIOECONOMIC STATUS, OCCUPATION AND HEV POSITIVITY (TABLE: 14)

Among the patients enrolled in the present study, only two patient was seen in lower socioeconomic status hence much infornation cannot be obtain. HEV positivity was more seen in the upper lower and lower middle socioeconomic status. According to Nargis et al socioeconomic status appeared to be the risk factor for IgG seroprevalence.

All seropositive cases were seen in the house wife. Because majority of the study population i.e., 91% were involved in house work (indoor). According to Alain B. Labriqueet al[56] in which he observed > 89% of the women reported their occupation as indoor housework.

OVERCROWDING AND FOOD HABIT WITH HEV POSITIVITY(TABLE: 15)

Majority of HEV positivity were noted in pregnant women residing in an overcrowded home. Overcrowding was statistically significant p value (0.002) with only IgM positive cases and both IgM+

& IgG positive cases (0.017). The above results coincides with the study done by Cosme et al, which states that hepatits E exposure was associated with overcrowding[55].

Overall, findings in this results put forward that, in overcrowded living conditions, hepatitis E virus transmission may be multifactorial and may include person to person transmission[57].

Majority of the pregnant women with HEV positivity were non-vegetarians. The above results shows that the asymptomatic pregnant women following Non-vegetarian dietary habit were predominantly associated with HEV seroprevalence. This high prevalence may be due to consumption of undercooked meat. According to Rakesh et al HEV-RNA has been detected from domestic swine in their faeces and also HEV antibodies have been detected in the sera of cattle, sheep, pigs and rodents[4].

DRINKING WATER AND USE OF BOILED WATER (TABLE: 16)

Majority of the HEV seropositivity were observed in pregnant women using pump water followed municipal water available inside their home for drinking purpose

Similar results was repoted by Zimaity et al in which patients living in homes that were not connected to a municipal source for drinking water appeared to have increased risk for HEV infection [58].

USE OF BOILED WATER:

Most of the HEV positive pregnant women were not using boiled water for drinking. This shows, it may be related to HEV positivity as boiling can inactivate HEV[5].

But from the above result, no positive association was found between source of drinking water and use of boiled water with HEV positivity. Similar observation was documented by Cosmeet al[55] and also agrees with results found in other studies[18].

TOILET FACILITY AND USE OF SOAP (TABLE: 17)

Among the pregnant women, majority of the seropositive cases were using common toilet (Indian type). Next most common was seen in cases who did not have toilet facility and were using open field for defaecation. Highly significant p value was found HEV seropositivity and toilet facility. Similar to the present study result, Surajudeen et al states that type of toilet was significantly associated with HEV seroprevalence and found to be more common among people who use open field for deafecation[59].

USE OF SOAP:

Most of the seropositive cases were noted in the pregnant women who did not have the habit of hand washing with soap after using toilet. It was highly significant statistically with the seroprevalence of HEV antibodies. Similar results was shown by Surajudeenet al[59].

CLINICAL DATA AND BLOOD GROUP (TABLE:18 AND TABLE:19)

Of the probable risk factors studied, antibodies to HEV was not associated with history of jaundice in the patients and family history of jaundice. There was no significant association with blood transfusion which was similar to the study done by Cosmeet al.[55]

NUCLEICACID AMPLIFICATION TEST

REVERSE TRANSCRIPTASE PCR

Nested PCR was done to all the IgM positive samples as IgM reaches peak titer in the first 4 weeks of infection after the onset of disease and disappears within 3 months in 50% of the patients [5]. IgG positive cases were excluded as they appears after IgM and persist for longer duration of time even upto 14 years[1,5]. Ninety negative cases were also

included in RT-PCR to look for the window period where the antibodies would not have mounted to a detectable levels.

Viremia will be first detected by RT-PCR, 22nd day after the exposure and over a week before onset of disease on 30th day[5]. A protracted period of viremia was also observed in small number of patients who were naturally infected[5].

All the IgM positive cases and 90 negative samples were found to be negative for HEV-RNA. This confirms that no patients were in the window period or in the active infection.

According to the sociodemographic characteristic with hepatitis E seropositivity in asymptomatic pregnant women, we found that hepatitis E virus exposure was positively associated with occupation, toilet facility, overcrowding and behavioral characteristics such as habit of hand washing with soap after using toilet and non-vegetarian diet.

Several of the risk factors assessed in the present study were interrelated. For illustration, a low level of educational level may be related with low hygiene practices such as not practicing the habit of hand washing after using toilet and no toilet facility may be associated with low socioeconomic status of pregnant women.

Seropositivity to hepatitis E virus infection was not statistically associated with age, gravida, source of drinking water , educational status, socioeconomic status and clinical data such as past history of jaundice in the patient, family history of jaundice, blood transfusion and blood group.

SUMMARY

Asymptomatic pregnant women attending routine antenatal check up in a tertiary care hospital were enrolled and the prospective study was conducted between “January 2014 and September 2014”. Total of 200 pregnant mothers were selected and evaluated by a questionnaire. ELISA and nucleic acid amplification test were done in the serum samples to detect anti-HEV antibodies HEV-RNA respectively.

- Majority (46%) of them were seen in the age group of 21-25 (mean age 24.55).
- Most of them were in the 2^{nd} and 3^{rd} trimester of pregnancy.
- Primigravida outnumbered second and multigravida.
- Educational status of most of the pregnant women (42.5%) was middle school.
- Most of the pregnant women (41.5%) belonged to lower middle class.
- 91% of the asymptomatic women were involved in indoor housework.
- Majority (69.5%) resided in overcrowded houses and (93.0%) followed non-vegetarian diet.

- Municipal water was the main source of drinking water and 75.7% do not use boiled water for drinking.
- Majority were using common Indian type toilet and 67% do not have the habit of hand washing.

HEV SEROPREVALENCE:

- In the present study, IgM and IgG seropositivity of asymptomatic pregnant women were **12 (6%)** and **18 (9%)** respectively.
- only IgM+ cases were **5 (2.5%),** only IgG+ cases were **11 (5.5%)** and both IgM+&IgG+ were **7 (3.5%)**.
- Most of the positive cases were in the age group 26-30.
- Seroprevalence was found to be statistically significant with overcrowding, type of toilet facility and habit of hand washing with soap after using toilet.
- Seropositivity was also found to be associated with increasing age, multigravida, pregnant woman involved in indoor housework.
- Seroprevalence was not associated with age, educational level, socioeconomic status, dietary habits, source of drinking water, use of boiled water, family H/O jaundice,

past H/O of jaundice in the pregnant women, history of blood transfusion and blood groups.

- When compared to the PCR results which was done for 90 negative cases and 12 IgM positive cases by ELISA, no cases were found to be positive for HEV-RNA. This confirms no viremia in the blood and hence no patients were in the window period or active infection.

CONCLUSION

In the present study, IgM and IgG seropositivity of asymptomatic pregnant women were **12 (6%)** and **18 (9%)** respectively. Among them only IgM+ cases were **5 (2.5%),** only IgG+ cases were **11 (5.5%)** and both IgM+&IgG+ were **7 (3.5%)**. Semi nested Reverse transcriptase PCR was found to be negative in all IgM positive.

From the present study we conclude that there is very low prevalence of anti HEV IgM and IgG antibodies among asymptomatic pregnant women and no cases were found to be positive for HEV-RNA or in the period of viremia. However these patients have to be screened periodically as they are high risk for developing fatal fulminant hepatic failure in their third trimester of pregnancy.

HEV-RNA testing can be considered in patients who are symptomatic for hepatitis E infection because viremia is the only marker of infection during the acute phase where anti HEV antibodies were not in a detectable levels. Also it should be considered in immunocompromised individuals as seroconversion could be delayed in these patients.

As hepatitis E virus spread through faeco-oral contamination, we should encourage public to adhere to the personal and household hygiene measures. Conservative control efforts at the community level such as provision of proper sanitation resources and water decontamination must also be complemented

Pregnant women are possibly susceptible to hepatitis E infection and are potentially high risk for fatal fulminant hepatic failure. Most recent trials of hepatitis E vaccine are certainly promising, and is found to be very efficacious in a controlled trial. Hence it can be considered in the most susceptible and high-risk groups in endemic countries.

PROFORMA:

DATE: MOBILE: BLOOD GP:

NAME: OP/IP NO:MICRO ID NO:

	1	2	3	4	5	6	7	8	9	10	11	12

AGE: EDUCATION: OCCUPATION:

ADDRESS:

SOCIO ECONOMIC STATUS:

NO. OF PEOPLE RESIDING AT HOME: SQ FT:

TOILET FACILITY: COMMON/ INDIVIDUAL

WATER FACILITY: BOILING WATER: YES/NO

PRACTICE OF HANDWASHING: YES/NO

COMPLAINTS:

GRAVIDA: LMP: EDD:

PAST OBSTERTIC HISTORY:

H/O: 1.BLOOD TRANSFUSION (YES/NO)

2. JAUNDICE (SELF/FAMILY)

TREATMENT HISTRORY:

IMMUNIZATION HISTORY:

OTHER TEST DONE:

GENERAL EXAMINATION:

PALLOR: YES/NO

ICTERUS: YES/NO

PHYSICAL EXAMINATION:

DATA SHEET

A	PC	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84
B	PC	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
C	NC	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
D	NC	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
E	IC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
F	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
G	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
H	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91

For 96 well MICROTITRE PLATE

PC- positive control

NC- negative control

IC- internal control

S- Samples

DS-EIA-ANTI-M

NATURE OF THE REAGENTS:

HEV Ag coated strips:

Polystyrene stripped 96 wells plate (breakable) coated with mix of recombinant antigens of HEV. Stored at 2-8° C until expiration date.

Sample diluent:

Transparent or slightly opalescent liquid, violet-blue coloured, sediment may form which completely dissolves at shaking. Preserving agent: 0.01% thimerosal. Store at 2-8° C until expiration date.

Conjugate concentrated 21-fold:

Monoclonal mouse antibodies against human IgM, labeled horseradish peroxidase. Transparent or slightly opalescent liquid, light yellow colored. Preserving agent: 0.04% ProClin 300, 0.04% gentamycin sulfate. Store at 2-8° C until expiration date in a tightly sealed vial

Conjugate diluent:

Transparent, yellow liquid at temperature of 2-8° C, opalescent yellow color liquid at temperature of 18-24°C. Preserving agent:

Preserving agent: 0.01% thimerosal. Store at 2-8° C until expiration date in a tightly sealed vial.

Positive control:

Heat inactivated human serum positive for anti-HEV-IgM, negative for anti-HIV-1,2, HBsAg and anti-HCV. Transparent or slightly opalescent liquid, red colored. Preserving agent: 0.04% ProClin 300, 0.1% sodium azide. Store at 2-8°C until expiration date in a tightly sealed vial.

Negative control:

Heat inactivated human serum positive for anti-HEV-IgM, negative for anti-HIV-1,2, HBsAg and anti-HCV. Transparent or slightly opalescent liquid, green colored. Preserving agent: 0.01% thiomersal , 0.1% sodium azide. Store at 2-8°C until expiration date in a tightly sealed vial.

Washing solution (concentrated 25 fold)

Transparent or slightly opalescent liquid, colorless, or pale yellow, sediment may form that dissolves at 35-39°C and shaking. Store at 2-8°C until expiration date in a tightly sealed vial.

Substrate buffer:

Citric acid and sodium acetate solution, pH 4.1-4.3, containing H_2O_2 . Transparent colorless liquid. Preserving agent: 0.04% ProClin 300. Store at 2-8°C until expiration date in a tightly sealed vial.

TMB concentrated 21- folds:

Solution containing Tetramethylbenzidine (TMB). Transparent colorless liquid. Store at 2-8°C until expiration date in a tightly sealed vial.

Stopping reagent:

0.2M/L sulphuric acid solution. Transparent colorless liquid. Store at 2-8°C until expiration date in a tightly sealed vial.

DS-EIA-ANTI-G

NATURE OF THE REAGENTS:

HEV Ag coated strips:

Polystyrene stripped 96 wells plate (breakable) coated with mix of recombinant antigens of HEV. Stored at 2-8° C until expiration date.

Sample diluent:

Transparent or slightly opalescent liquid, violet-blue coloured, sediment may form which completely dissolves at shaking. Preserving agent: 0.01% thimerosal. Store at 2-8° C until expiration date.

Conjugate concentrated 21-fold:

Monoclonal mouse antibodies against human IgG, labled horseradish peroxidase. Transparent or slightly opalescent liquid, light yellow colored. Preserving agent: 0.04% ProClin 300, 0.04% gentamycin sulfate. Store at 2-8° C until expiration date in a tightly sealed vial

Conjugate diluent:

Transparent, yellow liquid at temperature of 2-8° C, opalescent yellow color liquid at temperature of 18-24°C. Preserving agent:

Preserving agent: 0.01% thimerosal. Store at 2-8° C until expiration date in a tightly sealed vial.

Positive control:

Heat inactivated human serum positive for anti-HEV-IgG, negative for anti-HIV-1,2, HBsAg and anti-HCV. Transparent or slightly opalescent liquid, red colored. Preserving agent: 0.04% ProClin 300, 0.1% sodium azide. Store at 2-8°C until expiration date in a tightly sealed vial.

Negative control:

Heat inactivated human serum positive for anti-HEV-IgG, negative for anti-HIV-1,2, HBsAg and anti-HCV. Transparent or slightly opalescent liquid, green colored. Preserving agent: 0.01% thiomersal, 0.1% sodium azide. Store at 2-8°C until expiration date in a tightly sealed vial.

Washing solution (concentrated 25 fold)

Transparent or slightly opalescent liquid, colorless, or pale yellow, sediment may form that dissolves at 35-39°C and shaking. Store at 2-8°C until expiration date in a tightly sealed vial.

Substrate buffer:

Citric acid and sodium acetate solution, pH 4.1-4.3, containing H_2O_2 . Transparent colorless liquid. Preserving agent: 0.05% ProClin 300. Store at 2-8°C until expiration date in a tightly sealed vial.

TMB concentrated 21- folds:

Solution containing Tetramethylbenzidine (TMB). Transparent colorless liquid. Store at 2-8°C until expiration date in a tightly sealed vial.

Stopping reagent:

0.75 M/L sulphuric acid solution. Transparent colorless liquid. Store at 2-8°C until expiration date in a tightly sealed vial.

SEMI-NESTED PCR KIT FOR HEPATITIS E VIRUS

Kit component for 100 reactions

- RT-PCR master mix – 1ml
- Enzyme mix 100µl
- HEV/IC primer mix 990µl

- Internal control template 500µl
- HEV positive template 100µl
- Water, nuclease free 1ml

KEY TO MASTER CHART

- BLD GP- BLOOD GROUP
- UN EDU- UN EDUCATED
- HW- HOUSE WIFE
- NO OF PPL- NUMBER OF PEOPLE
- SQ FT- SQUARE FEET

- IND- INDIVIDUAL (INDIAN) TOILET
- OUT- OPEN FIELD DEAFECATION
- COM- COMMON TOILET (INDIAN TYPE)
- WESTR WESTERN TYPE OF TOILET
- 1R1H1K- 1 ROOM, 1HALL, 1 KITCHEN
- MUNI- MUNICIPAL WATER
- TAP OUT- COMMON TAP IN THE STREET
- G2P1- GRAVIDA1-PARA1
- LMP- LAST MENSTRUAL PERIOD
- EDD- EXPECTED DATE OF DELIVERY
- WOG- WEEKS OF GESTATION
- BLD TRANSFUSION- BLOOD TRANSFISION
- FAM HIST- FAMILY HISTORY OF JAUNDICE
- FOOD- FOOD HABIT
- NEG- GEATIVE

BIBLIOGRAPHY

1. The leading cause of acute viral hepatitis in the world

2. Seroprevalence of subclinical HEV infection in pregnant women from north India: A hospital based study-Nargis Begum, Salam Gyaneshwori Devi, Syed A. Husain- Received September 2, 2008

3. Min Liu1, Yili Chen1, Zhengyu Shen2 and Hongxu-Comparative clinical study on diagnostic detection of hepatitis E virus between nested polymerase chain reaction (PCR) and serological tests
August, 2013 African journal of Microbiology Research

4. Hepatitis E an over view and recent advances in clinical and laboratory research Journal of Gastroenterology and Hepatology

5. Fields text book of virology

6. Topley and Wilson text book of microbiology

7. P. Vasickova, I. Psikal, P. Kralik, F. Widen, Z. Hubalek, I. Pavlik- Hepatitis E virus: a review. VeterinarniMedicina, 52, 2007 (9): 365–384

8. T. P. E. Williams, C. Kasorndorkbua, P. G. Halbur, -Evidence of Extra hepatic Sites of Replication of the Hepatitis EVirus in a Swine Model
Journal Of Clinical Microbiology Sept. 2001, p. 3040–3046,

9.Malcolm Banks, Richard Bendall, Sylvia Grierson- Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 10, No. 5, May 2004

10. Nicole PAVIO1, Xiang-Jin MENG, Christophe- Zoonotic hepatitis E: animal reservoirs and emerging risks

11. Nicholas John Ashbolt - Microbial contamination of drinking water and disease outcomes in developing regions. Toxicology 198 (2004) 229-238

12. Robert H. Purcell and Suzanne U. Emerson- Hidden Danger: The Raw Facts about Hepatitis E Virus

13. MS Khuroo MD Riyadh, Saudi Arabia- DISCOVERY OF HEPATITIS E VIRUS - THE UNTOLD STORY

14. Mohammad Sultan Khuroo, SaleemKamii, ShahidJameel -Vertical transmission of hepatitis E virus

15. Patrick G. Halbur, ChaiyanKasorndorkbua, Xiang-JinMeng- hepatitis E virus

16.Scientific Committee on Enteric Infections and Foodborne Diseases Epidemiology and Prevention of Hepatitis E

17Toshinori Tanaka, Masaharu Takahashi, EijiKusano- Development and evaluation of an efficientcell-culture system for Hepatitis E virus

18.SerkanOncu ,SelcenOncu, Pinar Okyay- Prevalence and risk factors for HEV infectionin pregnant women

19. Dr.UdayakumarNavaneethan, MD, Dr.Mayar Al Mohajer, MD, - Hepatitis E and Pregnancy- Understanding the pathogenesis

20. A. Kumara,*, M. Beniwala, P. Karb, J.B. Sharmaa, N.S. Murthyc - Hepatitis E in pregnancy22 July 2003

21.Persistent Carriage of Hepatitis E Virus in Patientswith HIV Infection

22.The Two Faces of Hepatitis E Virus-22 July 2003

23. Andrew A Adjei, Yao Tettey1, John T Aviyase -Hepatitis E virus infection is highly prevalent among pregnantwomen in Accra, Ghana

24. Alain B. Labrique, Shegufta S. Sikder, Lisa J. Krain, Keith - Hepatitis E, a Vaccine-PreventableCause of Maternal Deaths

25. Silvia Sookoian-Symposium on liver &pregnancyLiver disease during pregnancy:acute viral hepatitis Annals of Hepatology 2006; 5(3): July-September: 231-236Annals of Hepatology

26.WHO Prevention &Controlof Viral HepatitisInfection:Framework for Global Action

27.Zakim and boyer's text book of liver diseases-Diagnosis and Detection of HEV

28. Padma billam et al- mechanism of pathogenesis and replication of an Avian strain of the hepatitis E virus in a chicken model.

29. HEV - background

30. Monica Mateus, ZekiaNeves, Ana Arauja-fulminant hepatitis E in a pregnant women-

31. Hepatitis E- august 2009 TRANSFUSION

32. Sandra Rodrigez, William D. Carey-Hepatitis E an evolving disease

33.Min Liu1, Yili Chen1, ZhengyuShenandHongxu Xu-Comparative clinical study on diagnostic detection of hepatitis E virus between nested polymerase chain reaction (PCR) and serological tests

34.M. Herremans,* E. Duizer, E. Jusic, and M. P. G. Koopmans-Detection of Hepatitis E Virus-Specific Immunoglobulin A in

Patients Infected with Hepatitis E Virus Genotype 1 or 3

35. Hepatitis E Virus (HEV) Strains in Serum Samples Can Replicate Efficiently in Cultured Cells Despite the Coexistence of HEV Antibodies: Characterization of HEV Virionsin Blood Circulation

36. Hepatitis E Virus Antibodies in Patients with Chronic Liver Disease

37. Identification of Genotype 3 Hepatitis E Virus (HEV) in Serum and Fecal Samples from Pigs in Thailand and Mexico, Where Genotype 1 and 2 HEV Strains Are Prevalent in the Respective Human Populations

38. Prevalence of Antibodies to Hepatitis E Virus in Veterinarians Working with Swine and in Normal Blood Donors in the United States and Other Countries

39. Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012

40. Use of Serological Assays for Diagnosis of Hepatitis EVirus Genotype 1 and 3 Infections in a Setting of Low Endemicity

41. Jun Inoue- Analysis of human and swine hepatitis E virus(HEV) isolates of genotype 3 in Japan that are only81–83 % similar to reported HEV isolates of thesame genotype over the entire genome

42. Czech Republic Petra Vasickova, Michal Slany, Pavel Chalupa, Michal Holub, Radek Svoboda, and Ivo Pavlik -Detection and Phylogenetic Characterization of Human Hepatitis E Virus Strains.

43. Detection and characterization of infectiousHepatitis E virusfrom commercial pig livers sold inlocal grocery stores in the USA

44.RakeshAggarwalÆShahidJameel- Hepatitis E vaccine

45. Pathogenesis and Treatment of Hepatitis E Virus Infection

46. Sporadic acute or fulminant hepatitis E inHokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food

47. Rabbit as a Novel Animal Model for Hepatitis E Virus Infection and Vaccine Evaluation

48. Swine Fact Sheet

49. Open reading frame structure analysis as a novel genotyping tool for hepatitis E virus and the subsequent discovery of an inter-genotype recombinant

50. Zoonotic hepatitis E: animal reservoirs and emerging risks

51. A serological immunoassay for Hepatitis E virus (HEV) diagnosis 1

based on genotype 3 Open Reading frame-2 (ORF-2) recombinant 2 proteins produced in Trichoplusianilarvae.

52. Challenges in diagnosis of Hepatitis E virus infections

53. Diagnosis of HEV infection by serological and real-time PCR assays: a study on acute non-A-C hepatitis collected from 2004 to 2010 in Italy

54. Serologic Assays Specific to Immunoglobulin M Antibodies against Hepatitis E Virus: Pangenotypic Evaluation of Performances

55. Cosme Alvarado-E sqivel, luis F - Hepatitis E virus exposure in pregnant women in rural Durango, Mexico.Annals of hepatology

56. Alain B. Labrique , * K. Zaman , Zahid Hossain , ParimalenduSaha , Mohammad Yunus-Population Seroprevalence of Hepatitis E Virus Antibodies in Rural Bangladesh

57.Christopher M .Howard , * Thomas Handzel , Vincent R . Hill , Scott - The American Society of Tropical Medicine and Hygien Novel Risk Factors Associated with Hepatitis E Virus Infection in a Large Outbreak in Northern

Uganda: Results from a Case-Control Study and Environmental Analysis

58. el-Zimaity DM[1], Hyams KC, Imam IZ, Watts DM, Bassily S, Naffea EK, Sultan Y, Emara K, Burans J, Purdy MA, et al- Acute sporadic hepatitis E in an Egyptian pediatric population.

59. Surajudeen A. Junaid1,2, Samuel E. Agina1 and Khadijah A. Abubakar- Epidemiology and Associated Risk Factors of Hepatitis E Virus Infection in Plateau State, Nigeria 2001 Jul-Sep;13(3):31-5.

60. Shams R[1], Khero RB, Ahmed T, Hafiz A-Prevalence of hepatitis E virus (HEV) antibody in pregnant women of Karachi

Informed consent form

Information to the subject

You are invited to participate in this study titled "**To Find The Prevalence Of Hepatitis E Virus Infection In Asymptomatic Antenatal Mothers Attending Routine Antenatal Check Up In A Tertiary Care Hospital**"

This study is an interventional one and it involves collecting of around 5 ml of blood and the general information about you and the facilities in your house.

This information is collected only for research purpose. The analysis will show the prevalence rate of Hepatitis E virus infection and the importance of screening, diagnosis and management about the infection. This study does not carry any risk to the participant. The benefit will be in the form of providing information and the importance of early detection, management and the possible outcome of the infection.

Participation in this study is purely voluntary. If u do not want to provide the information, you can withdraw from the study.

Refusal to participate will not affect your treatment.

Declaration by the subject

I, ---------- have been explained about the nature and purpose of the study by the investigator in the language that I best understood.

I have had the opportunity to ask questions about the study and the questions I had asked were answered to my satisfaction.

I know that my personal details will be kept confidentially, and I have the right to withdraw my consent at anytime.

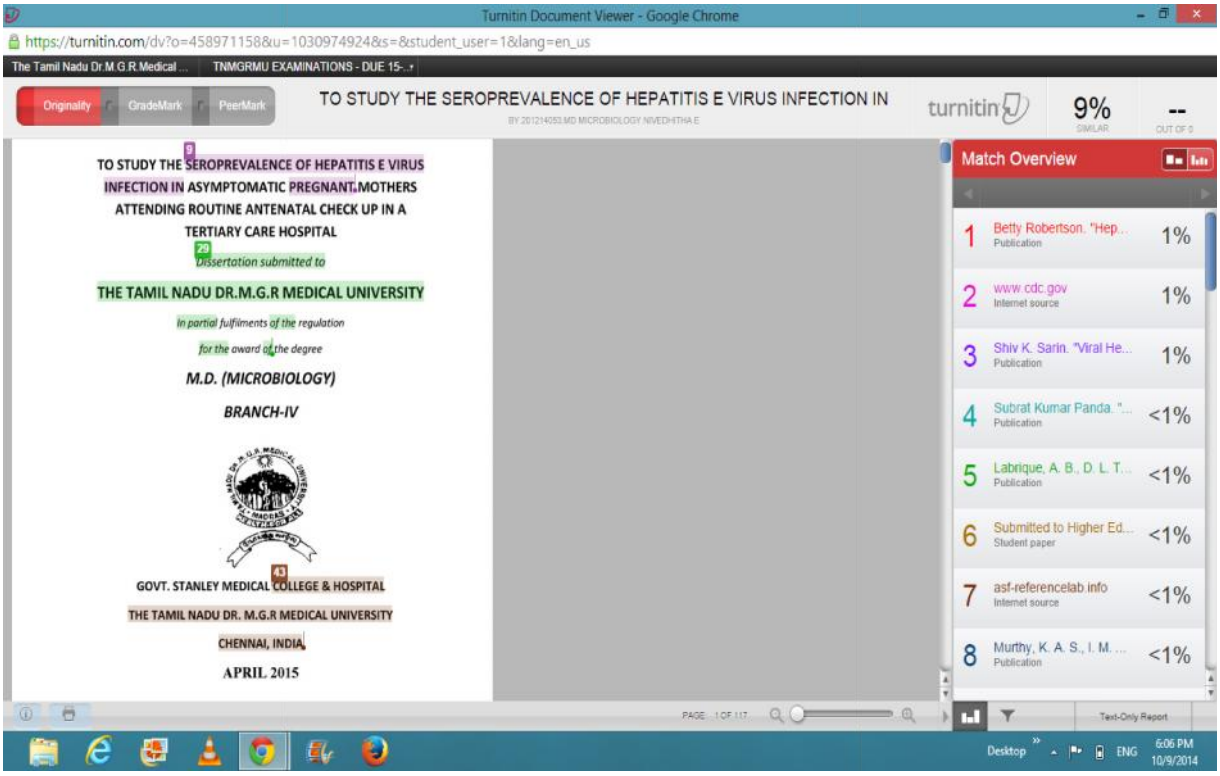
I consent voluntarily to participate in this study.

Name of the subject:

Signature / Thumb impression of the participant:

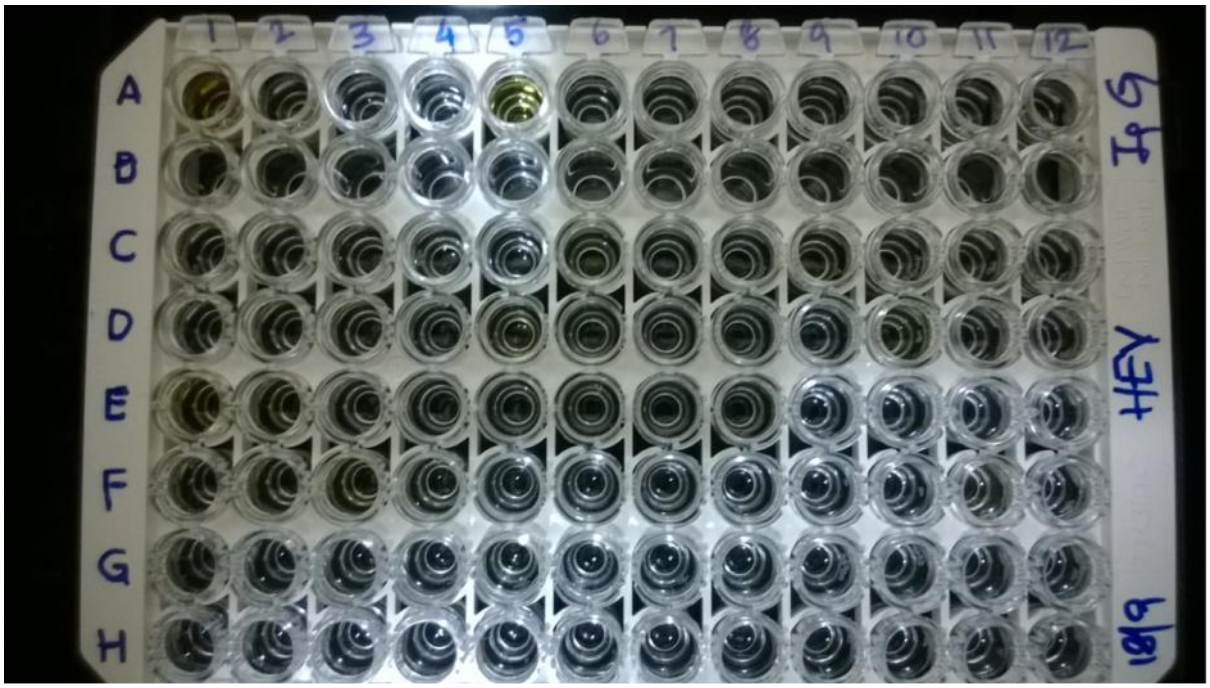
Name of the investigator:

Investigator's signature:


Turnitin Document Viewer - Google Chrome
https://turnitin.com/dv?o=458971158&u=1030974924&s=&student_user=1&lang=en_us
The Tamil Nadu Dr.M.G.R.Medical ...
TNMGRMU EXAMINATIONS - DUE 15-..
Originality
GradeMark
PeerMark
TO STUDY THE SEROPREVALENCE OF HEPATITIS E VIRUS INFECTION IN
turnitin
9%
SIMILAR
--
OUT OF 0
TO STUDY THE SEROPREVALENCE OF HEPATITIS E VIRUS INFECTION IN ASYMPTOMATIC PREGNANT MOTHERS ATTENDING ROUTINE ANTENATAL CHECK UP IN A TERTIARY CARE HOSPITAL
Dissertation submitted to
THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY
In partial fulfilments of the regulation
for the award of the degree
M.D. (MICROBIOLOGY)
BRANCH-IV
GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY
CHENNAI, INDIA.
APRIL 2015
Match Overview
1 Betty Robertson. "Hep... Publication 1%
2 www.cdc.gov Internet source 1%
3 Shiv K. Sarin. "Viral He... Publication 1%
4 Subrat Kumar Panda. "... Publication <1%
5 Labrique, A. B., D. L. T... Publication <1%
6 Submitted to Higher Ed... Student paper <1%
7 asf-referencelab.info Internet source <1%
8 Murthy, K. A. S., I. M. ... Publication <1%
Text-Only Report
Desktop
ENG
6:06 PM
10/9/2014

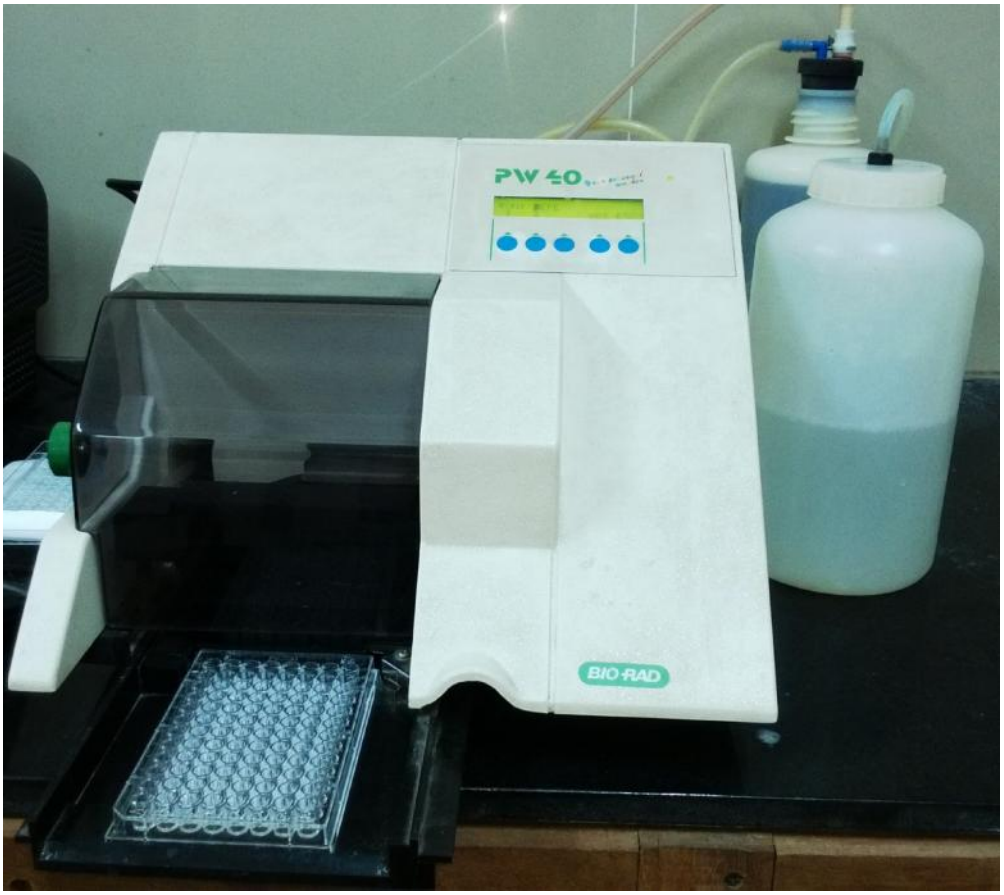
IgG – ELISA KIT

IgG – ELISA



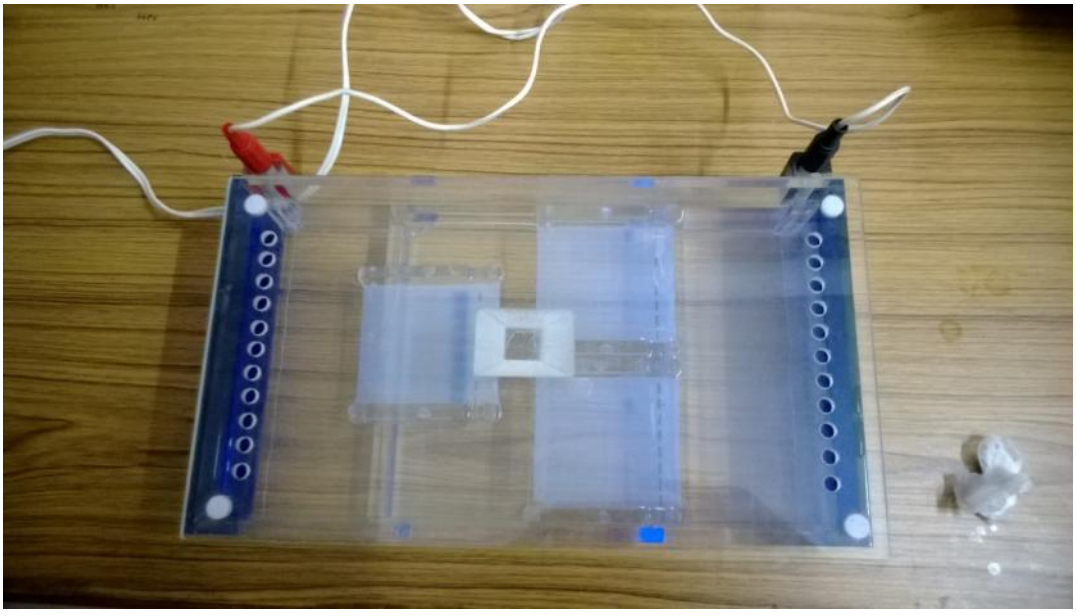

DSI
"DS-EIA-ANTI-HEV-M"
EMZYME IMMUNOASSAY FOR
THE DETECTION OF IgM
ANTIBODIES TO
HEPATITIS E VIRUS
LOT 040046
REF E-152
2014-12-30
Stopping Reagent
0.2M
25,0 ml
Sample diluent
12.5 ml
TMB
2.5 ml
2.5 ml
0.75 ml
Conjugate diluent
13.5 ml
Substrate Buffer
25,0 ml


A
B
C
D
E
F
G
H
HEV
1819
Nivi

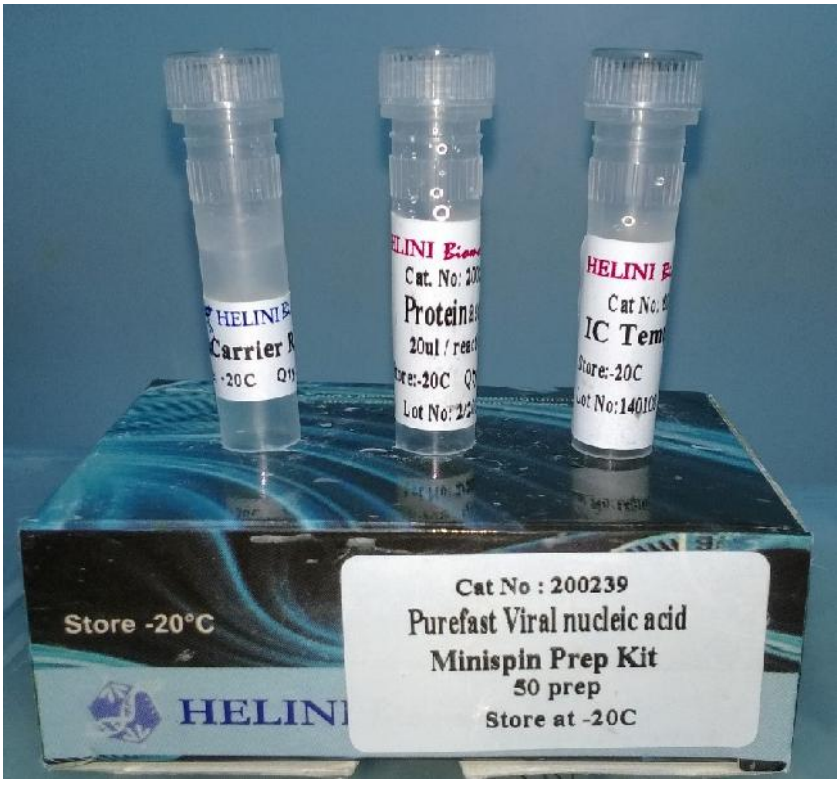

PW 40
BIO-RAD


BIO-RAD
Model 680
BIO-RAD


GLOBAL GENOMICS PARTNER
MyGenie96 Thermal Block
1
2
3
ESC
4
5
6
MENU
7
8
9
DEL
0
INS
ENTER
BIONEER

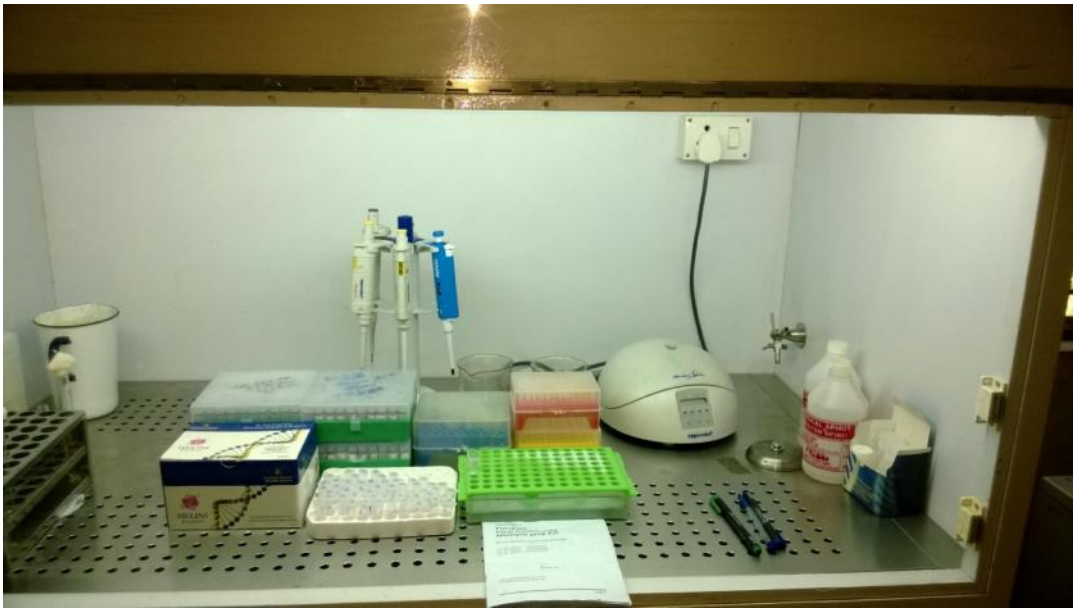



Gautham Pulavar
Molecular Research
In vitro Diagnostic
Real-Time PCR Kits & Reagents
HELINI
Biomolecules
Cat No : 200239
Purefast Viral nucleic acid
Minispin Prep Kit
50 prep
Store at Room temperature


Store -20°C
Cat No : 200239
Purefast Viral nucleic acid
Minispin Prep Kit
50 prep
Store at -20C


HELINI
HELINI
HELINI
Cat. No: 9
T PCR Mas
10ul / reacti
Lot No: 1/20
No: 9168
T Enzyme
l / reaction
Lot No:1/14
IC Primer
l / reaction
No: 1/2014
HEV
20C Qty:1
No: 0505


In vitro Diagnostic
Real-Time PCR Kits & Reagents
Molecular Research
HELINI Biomolecules
Cat. No: 200239/54
Spin column with
Collection tube
Store:RT Qty:25Nos
Lot No: 2/2014
Cat No : 200239
Purefast Viral nucleic ac
Minispin Prep Kit
50 prep
Store at Room temperat
HELINI Biomolecules
Cat. No: 200239
Flution Buffer
50ul / reaction
Store:RT Qty:
Lot No: 2/2014
HELINI Biomolecules
Cat. No: 200239
Lysis Buffer
100ul / reaction
Store:RT Qty:10ml
Lot No: 2/2014




miniSpin
Time
Speed x 1000
Start
Stop
Short
Spin
Open
eppendorf